REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

0132

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 12/10/97	3. REPORT TYPE AND DATES COVERED Final Technical Report, 01 Jun 94 - 30 Sep 97

4. TITLE AND SUBTITLE
Visualization, Identification and scaling of complex ecotoxicological dynamics at varying physical and temporal scales

5. FUNDING NUMBERS
USAFOSR Grant No. F49620-94-1-285

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Project - Task:2312//AS
Purchase Request No. : FQ8671-9400933
Authority: 10 U.S.C. 2358
61102F

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10. SPONSORING / MONITORING AGENCY REPORT NUMBER
AFOSR's CFDA #12.800

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

We have developed and confirmed experimentally the Community Conditioning Hypothesis. The hypothesis states that impacts to ecological structures are persistent, the variables containing information change over time, and ecological systems are irreversible. We confirmed persistence of effects in experiments ranging from 90 to 180 days for 3-L microcosms to 240 days for an outdoor mesocosm. Even wide differences in seasonal conditions or microclimate variation did not hide effects on larger systems. We conducted two types of experiments where microcosms were exposed to multiple stresses: (1) JP-8 stress was followed by heat shock and (2) two exposures 60 days apart to JP-8. For JP-8/heat, the initial stressor pattern was dominant with only minor changes in trajectory attributable to heat shock. For JP-8/JP-8, the second dosing was dominant. Thus, impacts due to multiple-stressor events can vary widely depending on timing and stress type. To aid analysis of effects at the community level, we developed a software package called MuSCLE that includes multivariate data analysis, pattern recognition, and visualization tools. We translated our results to the landscape level using metapopulation dynamic models. We found that impacts upon one patch can have dramatic effects upon other patches that are not contaminated.

QUALITY INSPECTED 4

19980205 050

14. SUBJECT TERMS
Outdoor mesocosm, JP-8, Community Conditioning Hypothesis, nonmetric clustering and association analysis, path dynamics, multiple stressors

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-1
298-102

Visualization, Identification and Scaling of Complex Ecotoxicological Dynamics at Varying Physical and Temporal Scales

USAFOSR

Grant No. F49620-94-1-0285

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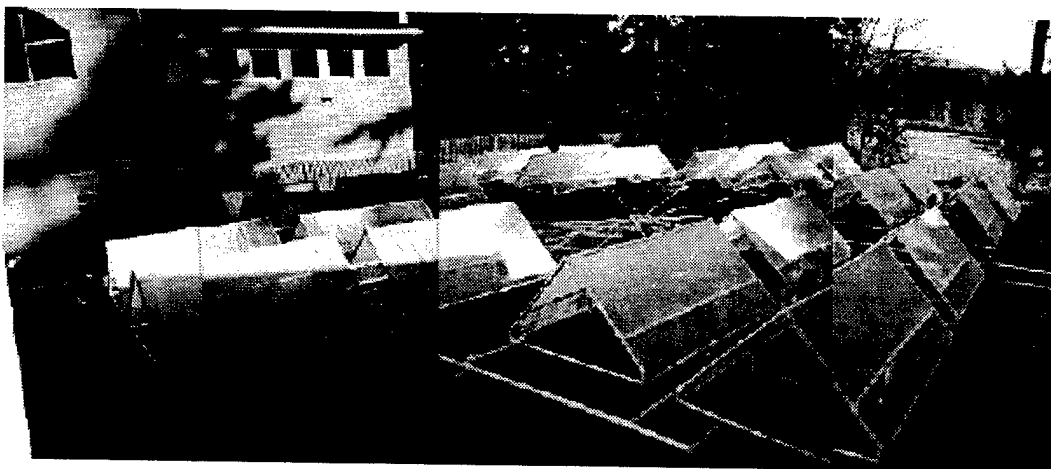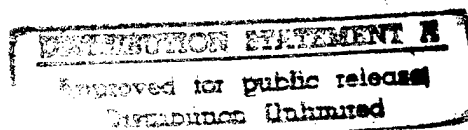
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Final Technical Report for June 1, 1994 through September 30, 1997

Prepared for
Life Sciences Directorate
Air Force Office of Scientific Research
Department of the Air Force
Bolling Air Force Base, DC 20332-6448

Institute of Environmental Toxicology and Chemistry
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19980205 050

Visualization, Identification and Scaling of Complex Ecotoxicological Dynamics at Varying Physical and Temporal Scales

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UNCLASSIFIED

Section 1-Overview and Summary

Abstract:

In course of this research and development program we have further developed and confirmed experimentally the Community Conditioning Hypothesis. This hypothesis states that the impacts to ecological structures are persistent, that the variables that should be measured change over time, and that ecological systems are irreversible. We have confirmed the persistence of effects in a series of experiments ranging from 90 to 180 days for 3 L microcosms to 240 days for our outdoor mesocosm system. Even wide differences in seasonal conditions or micro-climate variation in the outdoor mesocosm enclosure did not hide the effects of the JP-8 upon the larger systems.

We also conducted multiple exposure experiments where the microcosm system was exposed to a series of stresses. The first was a JP-8 stress followed by a heat shock, the second experiment was comprised of two exposures to JP-8 60 days apart. In the JP-8/heat experiment, the initial stressor was the dominant pattern with only minor changes in the trajectory that could be attributed to the heat shock with the variables being measured. In the second experiment, the dominant pattern at the end of the experiment was due to the second dosing with JP-8, with only hints of the initial pattern of dosing being apparent. These results demonstrated the impacts due to multiple stressor events can widely vary, depending upon the timing and type of stress.

A software package, MuSCLE was also developed to aid in the analysis of effects at the community level. The package is now Windows NT compatible and includes multivariate data analysis and pattern recognition tools as well as visualization software.

In addition, what we have learned has been translated to landscape level using metapopulation dynamic models that indicate the broad scope of toxicant impacts. Areas not containing toxicants may be impacted due to changes in the patterns of migration due to the toxicity within a patch. Reviewers of the paper now in press state that this research is a major contribution to the field of environmental toxicology.

The results of our research and the ranking approach in order to handle data has recently been applied to a risk assessment for the fjord of Port Valdez, AK. The proposed USEPA risk assessment guidance document also references our work in its discussion of recovery and the persistence of effects. Our data analysis tools have also been used in the elucidation of long term impacts due to the Exxon Valdez spill in Prince William Sound and in the examination of effluent data.

Key Words: Outdoor Mesocosm, JP-8, Community Conditioning Hypothesis, Nonmetric clustering and association analysis, patch-dynamics, multiple stressors.

Program Summary

We had several key objectives in the performance of this research proposal. They included:

1) A test of the Community Conditioning Hypothesis.
2) Long duration microcosm experiments were to be performed to test the persistence of the effects generated by the toxicant.
3) Development of an outdoor mesocosm in order to test the community conditioning hypothesis at larger scales.
4) Development of a set of software tools, multivariate software for community level ecology (MuSCLE) for conducting data analysis and visualization.
5) Extrapolation of our techniques and results to ecological risk assessment for the evaluation of toxicant impacts.

We have met each of these objectives and have continued to expand this research to include other indicators of toxicant impacts, the impacts of toxicants upon landscapes, and application of our research to regional ecological risk assessments. Our specific research accomplishments include:

1) Confirmation of the community conditioning hypothesis in two microcosm experiments of up to 180 days and in a mesocosm study of 240 days duration. The effects were persistent even in the outdoor system subject to seasonal variation in light and temperature.

2) Development of an outdoor mesocosm system for toxicity testing that ensures an equitable colonization of each system. Our unique arrangement of replicates and circulation strategy resulted in very low variance between replicates for critical parameters.

3) Completion of experiments where the microcosms were subjected to different stressors to simulate a more realistic scenario and to test the effects of prior stressors to the response of the systems to subsequent stressors.

4) Improvement of our data analysis software and completion of the transfer of the MuSCLE software to a Windows95/NT format. This should make the data analysis and visualization tools more available to the scientific community. The enclosed CD-ROM has a README file that contains the documentation for the program and our datafiles from our current experiments.

5) Modeling of the effects of toxicants upon patchy populations was begun and the first paper was accepted for publication. Using metapopulation and patch-dynamic theory, these models describe the interaction between a contaminated site and the uncontaminated sites all of which contain populations of the same species. We found that a contaminate in one site could dramatically affect the population dynamics in other sites if the populations are connected by migration. In addition, multiple outcomes may occur from the same set of initial conditions depending upon the distribution of the toxicant in the contaminated site.

6) We have now conducted two short courses during the annual meeting of the Society for Environmental Toxicology and Chemistry where we have presented the results from this research and its application to environmental toxicology and risk assessment. These courses have been among the best attended (sold out the last two years) of any of the short courses during the annual meetings.

7) We have applied a ranking methodology to an regional ecological risk assessment for Port Valdez, AK. In addition we conducted this risk assessment without using reference sites or assuming an equilibrium condition, lessons learned from our microcosm research. This ranking methodology and approach has already generated a great deal of interest in the risk assessment community.

8) Since the start of this project we have published 9 papers in peer-reviewed journals or as peer-reviewed book chapters and now have three in press for 1998. We have also given over 37 talks on the research, from local public groups to international scientific conferences. Six graduate students have received MS degrees.

The numbers of publications, technology transfers and collaborations are summarized in this section. Overviews of the various experimental and modeling projects are presented in the following sections.

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Masters Thesis Completed
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Presentations, 1994-1997

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Matthews, R. A. A. J. Markiewicz, V. L. Harter, and W. G. Landis , Incorporating Detrital Conditioning In Outdoor Microcosms Dosed With JP-8 Jet Fuel. 1997 Annual Meeting of the Society for Environmental Toxicology and Chemistry, Washington, D. C. November 16-20, 1997.

Graduate Students Supported by the Grant and Undergraduate Student Research Projects
Brown, Shiela. Development of the mesocosm protocol (J. Hardy-Huxley College)
DeMan, Michael. Database development for MuSCLE. Currently working for Apple, hopes to finish soon (G. Matthews-computer Science).
Gabrio, Rox-Computer programming and support.
Johns, Bettina- Derivation of the metapopulation models for toxicant patch dynamics (W. Landis-HuxelyCollege)

Harter, Virginia. Development of the mesocosm protocol (R. Matthews-Huxley College)
Kelly, Sue., Effects of multiple stressors on a multispecies system (W. Landis-Huxley College)
Macovsky, Louis. Development of the mesocosm protocol and patch-dynamic theory (W. Landis-HuxleyCollege)
Mortensen, Linda. Evaluation of Phytochelatin as a metal biomarker in the presence of JP-8 WSF. (W. Landis-Huxley College)
Pickreign, Cynthia. Riggle: a program for the dynamic conceptual time series analysis of hypervairate data and its application to ecotoxicology (G. Matthews-computer Science)
Rodgers, Sara - Comparison of MFC toxicity tests with and without adapted communities (Dr. Landis-HuxleyCollege).
Roze, Michael - Application of RIFFLE program for data evaluation (Dr. G. Matthews-Computer Science).
Spromberg, Julann A.- Derivation of the metapopulation models for toxicant patch dynamics (W. Landis-HuxelyCollege)
Wilson, Valerie. The impact of soil composition on the earthworm, *Eisenia foetida*, response to a hydrocarbon toxicant (W. Landis-Huxley College)

Professional Collaborators in the Research Program
Frieda B. Taub, School of Fisheries, University of Washington, Seattle, WA
John H. Taub, Seattle, WA.
Anne Fairbrother, ecological planning and toxicology, Corvallis Oregon.

Interactions and Consultations
     Over the last year this research has been translated into technology transfers to DOD and EPA laboratories and the private sector. Apart from presenting the research at national and international meetings, we have been successful in transferring this data and technology during informal meetings or presentations on-site. Below is a list of several of the groups with which we met and transferred information over the last 36 months.

Lidia Watrud, Team Leader, and Ray Siedler Biotechnology Team, U.S. EPA-Corvallis, OR. Data analysis from terrestrial microcosms.

Nigel Blakley, Department of Ecology, Olympia, WA. Toxicity evaluation of petroleum mixtures.

Zeneca. Data analysis of aquatic microcosm studies.

Anne Sergeant, ORD, U.S. EPA., Washington, D.C. Application of multivariate methods to ecological risk assessments.

Scott Ferson, Applied Biomathematics, Setauket, NY. Nonmetric clustering techniques.

Technology Transfers
USEPA Corvallis, Lidia Watrud. Use of NCAA for the determination of patterns in soil microbial communities.

SETAC short courses, 1996-1997. Community conditioning, metapopulation dynamics and the use of multivariate statistics in the analysis of environmental data.

Peter Chapman, Zeneca. The use of NCAA in the analysis of mesocosm data sets.

Prince William Sound Regional Citizens' Advisory Committee. Use of ranking methods in ecological risk assessment.

Metapopulation Dynamics, Indirect Effects, Multiple Discrete Outcomes and Uncertainty in Ecological Risk Assessment.

W. G. Landis, and B. Johns Institute of Environmental Toxicology and Chemistry, Huxley College of Environmental Studies, Western Washington University, Bellingham, WA., J. A. Spromberg, Toxicology Program, University of Kentucky, Lexington, KY.
A critical issue in environmental toxicology is the impact of xenobiotics at different scales. Metapopulation dynamic models are tools for investigating the potential for impacts of a contaminant upon the organisms within a patchy landscape. In our current series of simulation studies we have investigated the impact of the initial population sizes, distances between patches, and dose-response curves upon the dynamics of each subpopulation. The models themselves are discrete, deterministic and exhibit density dependence upon growth and immigration rates. All patches are equivalent habitats except that one patch is contaminated. An assumption is also made that the distribution of the toxicant is contagious and we use a Poisson distribution to simulate exposure. Persistent and degradable toxicants were modeled. As a toxicant degrades several discrete outcomes are available. The outcomes for a single patch and initial conditions can range from extinction to reaching carrying capacity or exhibiting bifurcating dynamics. Variation among multiple runs for a single patch is largest at the end of the simulation. Small changes in initial population size, patch distance or patch arrangement can drastically change the probabilities of extinction, reaching carrying capacity or the onset of bifurcating dynamics. The output has important implications for environmental toxicology and risk assessment. Multispecies toxicity tests and field data should also be examined for impacts upon non-stressed populations in a patchy environment. Impacts upon non-stressed subpopulations have been observed in fisheries biology. Additionally, the same stressor may result in different and discrete outcomes, with the probabilities changing in a nonlinear fashion. This research is supported by USAFOSR grant F49620-94-1-0285.

Incorporating Detrital Conditioning in Outdoor Microcosms Dosed with JP-8 Jet Fuel.

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We have developed an outdoor microcosm that incorporates detrital conditioning to test the hypothesis that microbiota play a critical role in altering the community response to hydrocarbon toxicants. The microcosms were constructed using 568 L tanks, arranged in 6 units of 4 tanks, with each unit equidistant from a central conditioning tank (CT). During pre-treatment, the microcosms and CT were filled with nutrient-amended well water (500 μg-N/L and 20 μg-P/L), artificial sediment (silica sand, ground chitin, and cellulose powder) leaf packs containing dried maple leaves, elodea fragment, and unglazed tiles for periphyton growth. Water circulation was maintained at the rate of 24 exchanges per day. After four weeks, invertebrates from local ponds were added to the CT. Leaf packs were added to the CT and microcosms every two weeks; eight week old packs were discarded after returning invertebrates to the CT. On a weekly basis, 25% of the sediments, leaf packs, tiles, and elodea from each microcosm were transferred to another microcosm; the CT walls and tiles were scraped; and the water quality was monitored. Circulation was discontinued one week prior to dosing. On 4/12/96, the microcosms were dosed to contain 0-0.25 μg/L of JP-8 jet fuel. Within two weeks the GC/MS hydrocarbon concentrations were very low in the water column of the highest treatment group. There has been little acute toxicity, despite selecting doses that caused sever, acute toxicity in laboratory microcosm studies. The presence of a complex, detritus-based microbial community appears to mitigate the influences of the toxicant on the microcosms.

The Impacts of Multiple Stressors to Model Ecological Structures.

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Institute for Watershed Studies, Huxley College, G. B. Matthews., Computer Science Department, Western Washington University, Bellingham, WA. The basis of the community conditioning hypothesis is that ecological structures are the result of their unique etiology. Systems that have been exposed to a variety of stressors should be reflect this history. We have now conducted a series of microcosm experiments that can compare the effects of multiple stressors upon community dynamics. The microcosm protocols are derived from the Standardized Aquatic Microcosm (SAM) and have *Lemma* and additional protozoan species. Two multiple stressor experiments have been conducted. In an extended length SAM (ELSAM), two of four treatments were dosed with the turbine fuel JP-8 one week into the experiment. Two treatments were later exposed to the heat stress, one that had received jet fuel and one that had not. Similarly, an ELSAM was conducted with the second stressor being the further addition of JP-8 replacing the heat shock. Biological, physical and chemical data were analyzed with multivariate techniques including nonmetric clustering and association analysis. Space-time worms and phase diagrams were also employed to ascertain the dynamic relationships of variables identified as important by the multivariate techniques. The experiments do not result in a simple additive linear response to the additional stressor. Examination of the relative population dynamics reveal alterations in trajectories that suggest treatment related effects. As in previous single stressor experiments, recovery does not occur even after extended experimental periods. We are now attempting to measure the resulting trajectories, changes in similarity vectors and overall dynamics. However, community conditioning does appear to be an important framework in understanding systems with a heterogeneous array of stressors.

Society of Environmental Toxicology and Chemistry Annual Meeting, San Francisco, CA November 16-20 1997.

Impacts of JP-8 jet fuel on microbial and macroinvertebrate communities in aquatic microcosms. R. A. Matthews, Institute for Watershed Studies, W. G. Landis, and A. J. Markiewicz, Institute of Environmental Toxicology and Chemistry, and G. B. Matthews, Computer Science Department, Western Washington University, Bellingham, WA. We investigated the impacts of neat JP-8 jet fuel on microbial and macroinvertebrate communities in 380 L outdoor aquatic microcosms. Twenty-four 380 L microcosms were constructed around a 12,000 L central conditioning tank (CT). Prior to dosing the microcosms and CT were filled with nutrient-amended water, artificial sediments, leaf packs, Elodea fragments, and unglazed tiles. Water was circulated (24 exchanges/day), invertebrates from local ponds were added to the CT, and 25% of the sediments, leafpacks, tiles, and Elodea were transferred between mesocosms every two weeks. The water quality and biota in the microcosms were sampled 25- and 3-days prior to dosing, and weekly for 8 months after dosing.
On 12-April-96 the microcosms were dosed with 0-95 mL of neat JP-8. These doses had very little toxic effect, despite yielding water column hydrocarbon concentrations that should have been acutely toxic to invertebrates. During the first two weeks, the hydrocarbons appeared to stimulate bacterial growth, seen as increased microbial respiration. After the initial response, the major patterns were seasonal responses to changes in light and temperature rather than dose-response effects. The presence of a complex, detritus-based microbial community appears to mitigate the influences of the toxicant in the microcosms.

The Impact of Soil Composition on the Earthworm, *Eisenia foetida*, Response to a Hydrocarbon Toxicant. V. J. Wilson, W. Landis, Institute of Environmental Toxicology and Chemistry, Western Washington University, Bellingham, WA. Formulations of artificial soil used in earthworm toxicity tests include sand, clay and peat moss components which may affect the bioavailability of the toxicant. These components may partially immobilize the toxicant thereby increasing the concentration at which earthworms exhibit lethal responses. Lethal effects on the compost worm, *Eisenia foetida*, exposed to concentrations of jet-fuel are determined using

artificial soil of different compositions. Artificial soil is made up of varying proportions of sand, organic material (peat moss) and clay.

The newly developed ASTM test methodology for determining earthworm toxicity was used to develop concentration-response curves for artificial soil with high amounts of clay and high amounts of organic material. Results indicated that the proportions of soil components do impact the earthworm response to the jet-fuel toxicant. Soil with increased peat moss and decreased clay components exhibited a concentration-response curve that indicated a lower toxicity to earthworms than standard ASTM artificial soil. In soil with increased peat moss (with the clay component equivalent to the standard soil), the toxicity, as indicated by the concentration-response curve, varied according to the amount of peat content. In soil with increased clay, there were no significant differences in the concentration-response curves as compared to ASTM standard soil. These changes in soil toxicity may reflect differences in soil textures and soil chemistry between various soil types.

Community Conditioning and Experimental Design Parameters for Aquatic Multispecies Toxicity Tests. W. G. Landis, A. J. Markiewicz, Institute of Environmental Toxicology and Chemistry, R. A. Matthews, Institute of Watershed Studies, Huxley College, G. B. Matthews, Department of Computer Science, Western Washington University, Bellingham, WA. We have conducted multispecies toxicity tests with three very different protocols, the mixed flask culture (MFC), the standardized aquatic microcosm (SAM), and an outdoor mesocosm. In addition we have developed the community conditioning hypothesis to describe the features and dynamics that we have observed in these experiments and in field studies. We are proposing design parameters that could be utilized in a variety of multispecies toxicity tests to aid data collection, analysis, and interpretation. A few of these design considerations are:

1. Multispecies toxicity test systems are complex, non-equilibrium, historical, and non-linear. Ecological structures do not recover or return to thier original state.
2. Every care must be made to ensure the similarity of the experimental replicates. This includes introducing known components or ensuring that the migration pathways from the source are equal for all replicates.
3. Environmental gradients require a random block experimental design.
4. Avoid pseudoreplication. This means multiple samples from the same enclosure cannot ever be treated as experimental replicates.
5. Univariate statistical techniques are not appropriate for multivariate structures.
6. Calculation of NOECs and LOECs are inappropriate.
7. Multivariate methods are more suitable for the data analysis of multispecies toxicity tests. No one multivariate technique is always best.
8. Data exploration using a variety of techniques should be followed by confirmatory statistics.
9. Multivariate visualization techniques should be used.

Evaluation of Phytochelatin Production as an Exposure Biomarker for Metals Through Laboratory Testing of Algae. L. S. Mortensen, and W. G. Landis, Institute of Environmental Toxicology and Chemistry, Huxley College of Environmental Studies, Western Washington University, Bellingham, WA. Phytochelatins are sulfur rich polypeptides found in plants from all major families and have the general structure of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ where n= 2 to 11. Functionally similar to mammalian metallothioneins, their formation in plants is induced by the presence of heavy metal ions. Recently, phytochelatins have been proposed as biomarkers of heavy metals in field studies. Contaminated environments most often contain waste mixtures rather than single compounds. This study examined phytochelatin induction in the green algae *Selenastrum capricornutum* by 48 hour exposure to cadmium, lead and the water soluble fraction (WSF) of JP-8 jet fuel. Phytochelatin induction by mixtures of cadmium and JP-8 WSF, and cadmium and lead were also tested. Finally a complex mixture, the WSF of crude oil, assumed to contain cadmium and lead as impurities, was tested for phytochelatin induction. Cadmium and lead

induced phytochelatin production while JP-8 and crude oil WSFs did not. Cadmium and WSF JP-8 fuel mixture tests resulted in slightly increased phytochelatin production compared to phytochelatin production in tests using only cadmium. Cadmium and lead in mixture were antagonistic in terms of phytochelatin production compared to phytochelatin production in tests using cadmium alone. Phytochelatin measurement appears to be a good exposure biomarker because it is sensitive and specific for metals and not inhibited by common organic pollutants.

Section 2. A Test of the Community Conditioning Hypothesis: Persistence of Effects in Model Ecological Structures dosed with the Jet Fuel, JP-8.

Introduction

In our previous studies (Landis et al 1993a, 1993b, 1995, Matthews Landis and Matthews 1996) we have observed the persistence of effects with a series of microcosm toxicity tests using a variety of turbine fuels. These effects persist long after the toxicant has degraded or otherwise been eliminated from the microcosm. These observations are inconsistent with a disturbance followed by recovery model. We have proposed and alternative model, the community conditioning hypothesis, which states that ecological communities tend to preserve information about every event in their etiology (Matthews, Landis and Matthews 1996, Landis, Matthews and Matthews 1996). The basic components of this hypothesis follow.

- Communities are a product of their unique etiology, which is the historical collection of physical, chemical and biological events leading up to a point in time. No two ecological systems will ever be identical.

- Events that alter the structure or function of populations within the community become a part of the history of the community and are difficult to erase. The influence of the event may increase or decrease over time, but is not lost from the history of the community.

- Information can be stored in an array of biotic and abiotic forms, such as varieties of detritus types, phenotypic fitness or change in age structure. Any subset of community measurements, such as single-species population counts or reproduction dynamics, cannot be assumed to be representative of the entire community.

- Information may be retained by properties of the community that remain hidden for indefinite time periods. The potential of this conditioning to alter the future trajectory of the community may remain undiminished.

  Almost all environmental events leave lasting effects. No **observed** effect does not mean no effect, the observer may just be looking in the wrong place, not have used appropriate data analysis tools or too small a sample size.

- The search for the recovery of an ecological structure in meaningless in terms of the ecological system. Recovery by definition (Lewontin 1969) eliminates the history of the system. Only in terms of human value structure expressed as a subset of ecological variables can the illusion of ecological recovery be observed.

This paper presents a series of comprehensive studies detailing the biological and ecological impacts of the water soluble fraction (WSF) of the turbine fuel JP-8. These studies are designed to compliment the previously published research examining the impacts of Jet-A and JP-4 (Landis et al 1993a, 1993b) using the Standardized Aquatic Microcosm (SAM).

We used a modification of SAM in these experiments not to mimic a particular aquatic system, but as a model that has some of the properties of naturally derived ecosystems. These properties include food web structure, a variety of photosynthetic and grazer organisms, and the property of being a complex system. No constructed ecological system is ever going to replicate a natural system since they will always have dissimilar histories of colonization and artificial systems are much more closed to migration and immigration (Landis et al 1997). However, these constructed systems are useful research tools in examining particular features and properties of naturally occurring systems. Our goal in this study was to examine the persistence of historical events within a ecosystem using the SAM as a model.

In the experiments reported here the effects of the water soluble fraction (WSF) of the turbine fuel JP-8 were observed in a set of single species and then in two multispecies toxicity test experiments. The multispecies experiments were based on the Standardized Aquatic Microcosm (SAM) protocol. One SAM experiment was at the normal 63 day length, the other was twice a long. In both experiments clusters associated with treatment persisted during the experiment after the degradation of the WSF. Although the patterns observed in each experiment were similar, the specifics were not. The variables that best described each system changed with sampling date, although broad similarities could be found. The importance of historical events, the irreversibility of ecological systems and the contrasting viewpoint of this research is contrasted with the search for recovery as an endpoint.

Methods
Chemicals

All chemicals used in the culture of the organisms for the Standardized Aquatic Microcosm and in the preparation of the microcosm medium, T82MV, were reagent grade or as specified by the ASTM and USEPA protocols. Individual hydrocarbon reference standards, that were used to identify and quantify the water soluble components in the jet fuels, were purchased from the Alltech Chemical Company (Deerfield, IL), were certified to 99+% purity and A.C.S. spectrophotometric grade. The ASTM D3710 Qualitative Calibration Mix and the Qualitative Reference Reformate Standard were purchased from Supelco Chromatography Products (Bellefonte, PA). All standards were prepared in pesticide residue grade, A.C.S. specification hexane or carbon disulfide, purchased from VWR Scientific (Seattle, WA).

JP-8 was supplied by the U. S. Air Force Toxicology Laboratory at Wright Patterson Air Force Base in Ohio. The samples were collected in two liter fuel cans from in-line quality assurance/quality control valves, sealed on site, lot shipment recorded and transported to the laboratory, using in-place chain-of-custody procedures. The procedure for producing the water soluble fraction (WSF) of a turbine has been described (Landis et al 1993b).

Algal and Daphnid Toxicity Tests

In order to estimate the short term and relative toxicities of the JP-8 WSF mixture a series of short-term toxicity tests were performed. These included the 96 hr algal growth inhibition with three species of algae and the 48h *D. magna* toxicity test.

Algal growth inhibition: Algal growth inhibition tests were performed to determine the toxicity of the WSF of the various fuels using *Chlorella vulgarius, Ankistrodesmus falcatus* and *Selenastrum capricornutum*. Test algae were grown in a semi-flow through culture apparatus on the microcosm media T82MV and taken during log phase growth for inoculation into the test flasks. Five hundred mL Erlenmeyer flasks with ground glass stoppers were used as test chambers. with serial dilution's of the water soluble fraction at concentrations of 0.0, 6.25, 12.5, 25, 50 and 100 percent then placed in the flasks. The test organisms were added at a concentration of approximately 3.0×10^4 cells/mL. Total volume was 100 mL with two replicates of controls and the test concentrations used. Test mixtures will be incubated at $20.0°C \pm 1.0°C$ with a 12:12 hour light/dark cycle. Using a Newbauer Counting Chamber, cell densities were determined every 24 hrs for the 96 hr duration of the test.

The cell numbers are then plotted against the concentrations. If possible a least square regression line was drawn and the IC_{50} (the concentration at which algal growth is reduced to 50% of the control) determined.

D. magna toxicity test: *D. magna* acute toxicity tests were conducted using T82MV media at concentrations of 0.0, 6.25, 12.5, 25, 50 and 100 percent WSF. Ten neonates were placed in each 250 mL beaker containing 200 mL of test solution. Two replicates were used at each concentration. At 24 and 48 hrs the number immobilized was recorded. Data were analyzed graphically and statistically to obtain an estimate of the EC_{50}.

Gas Chromatography of WSF

A Tekmar LSC 2000 Purge and Trap (P&T) concentrator system in tandem with a Hewlett Packard 5890A Gas Chromatograph with a Flame Ionization Detector (FID) was used for the analysis of all microcosm samples and standards (Landis et al 1993b). Instrument blanks and deionized distilled water blanks are used to verify the P&T and GC columns cleanliness prior to analysis of samples. A 5 mL gas tight Teflon Luer lock syringe was used to remove a 3.5 mL sample and inject it into the 5 mL sparger where the sample was purged with pre-purified nitrogen gas for eleven minutes and dry-purged for four minutes. Volatile hydrocarbons, purged from the sample and collected on the Tenax/Silica Gel column, were desorbed at 180°C directly onto the gas chromatograph SPB-5, 30 m x 0.53 mm ID 1.5 μm film, fused silica capillary column. The GC column was programmed to hold at 35°C for two minutes, increase to 225°C at 12°C/min and hold at that temperature for five minutes. A Spectra-Physics 4290 Integrator recorded the FID signal output of the volatile hydrocarbons, separated and eluted from the column by molecular weight and boiling point. A comparison was then made of the sample chromatograph peak retention times and area under the peak curve to n-paraffin and aromatic chromatograph reference standards, prepared and analyzed under the same conditions, for sample concentration determinations.

Multispecies Toxicity Tests
The 63-day SAM protocol previously has been described (ASTM E 1366-91, 1991) and a summary describing the use of the technique in the testing of turbine fuel has been published (Landis et al 1993b). The experimental design consists of a defined ecological system with a specified algal, invertebrate and microbial community. Four treatment groups of 6 independent replicates were used.
Two major modifications were made to the SAM protocol. The first was the means of toxicant delivery. On day 7, 450 mL were removed from each container using an autoclaved, 100 mL capacity basting tube, with a sterile square of 100 mesh Nitex® tied over the opening to prevent the removal of the organisms. The 100% WSF stock material was then combined with fresh, sterile T82MV and added in appropriate amounts to produce concentrations of 0, 1, 5 and 15 percent WSF for the four treatment groups (Treatment 1-4 respectively). After toxicant addition the final volume was adjusted to 3L. The second modification was the substitution of *Tetrahymena thermophila* BIV for the hypotrichous ciliate used in past experiments. The results presented below demonstrate the suitability of the Tetrahymena for inclusion in the protocol. The microcosms were monitored for structural parameters, with subsamples removed from each microcosm and counts of population densities made for all species, on Tuesdays and Fridays, for the duration of the experiments.
In order to examine the repeatability of a microcosm , two separate experiments were performed in the same environmental room, using the same cultures, and with the measurements taken by essentially the same staff. The first experiment, JP-8-1, was for 63 days. Immediately following the JP-8-1 experiment, a 126 day experiment was performed , JP-8-2.

Data analysis
Data were collected and analyzed in a conventional fashion as directed by the protocol. Intervals of non-significant difference (α =0.05) were calculated and plotted for the measured variables. Shannon-Weaver diversity was calculated for the algal assemblage.
Three multivariate techniques were used to identify patterns and compare the clusters to treatment groups. Two of them were based on the ratio of multivariate metric distances within treatment groups vs. between treatment groups. One of these is calculated using Euclidean distance and the other with cosine of vectors distance (Good, 1982; Smith *et al.*, 1990). The third test used nonmetric clustering and association analysis (NCAA) (Matthews and Hearne 1991, Matthews et al 1991a, 1991b, Matthews et al 1995). Redundant measures such as algal biovolume and algal species diversity based on the algal counts and were eliminated from the clustering.

The approach to using NCAA has been modified to emphasize the optimization of cluster quality. This process is conducted in an iterative fashion. Generally, the number of clusters being sought for is reduced, the number of iterations increased to improve the chance of finding a maximum clustering, and the numbers of best variables being identified is reduced. Using the graphical output and the measures of cluster quality it can be determined that cluster quality is approaching at least a local maximum. The optimal clusters are then compared to treatment groups with an association test and the significance determined. We will present the results of different combinations of cluster quality, number of important variables and the results of the Chi-square goodness of fit test.

Results

Single species toxicity tests

The results of the single species toxicity test results conducted with the WSF of JP-8 are presented in Table 1 and Figure 1. In some cases multiple toxicity were conducted with the same test species and those results are shown individually. Generally the IC50 for the three algal species tested, Ankistrodesmus, Selenastrum and Chlamydamonas, was higher than the EC50 results for *D. magna* and *D. pulex*. This is a pattern common with the other turbine fuels tested to date (Landis 1993a, 1993b). Compared to results for the WSF of crude oil, the toxicity is in the same range.

The test data are represented as scatter plots in Figure 1. Variability in the daphnid toxicity tests was relatively low, and reflects the shallow concentration-response curve. The *D. pulex* results were within the range of data for *D. magna* (Figure 1a). There was much more scatter apparent in the data obtained for the three algal species tested (Figure 1b). Again there was a shallow concentration-response relationship for JP-8.

Fate of JP-8 in Microcosms

The constituents of the WSF were rapidly removed from the water column. Figure 2 depicts two chromatograms taken from a treatment 4 replicate of SAM JP-8-1. At the initial dosing of the container a variety of materials were visible and in high concentration. Forty-eight hours later the area under the curves were substantially reduced. In order the quantify the removal rates, specific compounds were followed during the course of the SAM experiment and two of these, benzene and toluene, are presented in Figure 3.

Benzene (Figure 3a) was rapidly removed from the water column, with half of it removed by 72 hours after dosing. The downwards trend continues until after 192 hours (8 days) an increase in noted in the concentration of the material. This is not due to the recurrent mixing of the sediments and other components of the microcosm, since that had occurred several times before.

Toluene was present at five times the concentration of benzene (Figure 3b). At 72 hours over two thirds of the material was removed from the water column. The removal appeared to be asymptotic after 144 hours (6 days), yet the material increased in concentration after 240 hours post dosing. Again, the increase does not appear to be directly related to the stirring of the microcosm during sampling.

JP-8 Toxicity in 48 hr Daphnia Static Toxicity Test

A

Legend:
- □ *D. magna*-1
- ▲ *D. magna*-2
- ○ *D. magna*-3
- ■ *D. pulex*

96 hr Algal Inhibition Tests JP-8

B

Legend:
- □ Selenastrum
- △ Ankistrodesmus-1
- ○ Chlamydamoras-1
- ▲ Ankistrodesmus-2
- ● Chlamydamoras-2
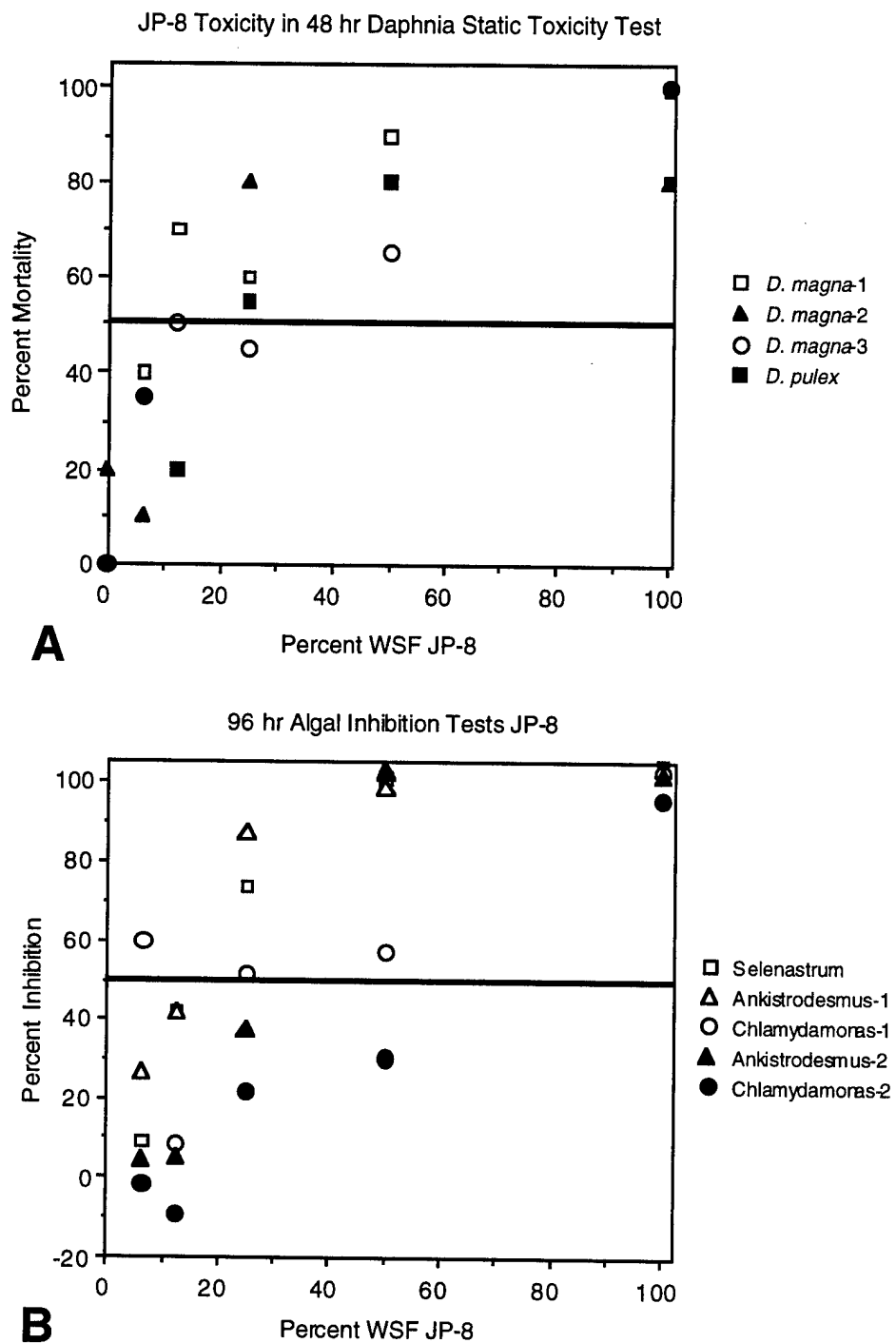
Figure 1. The results of the single species toxicity test results.

A   **B**
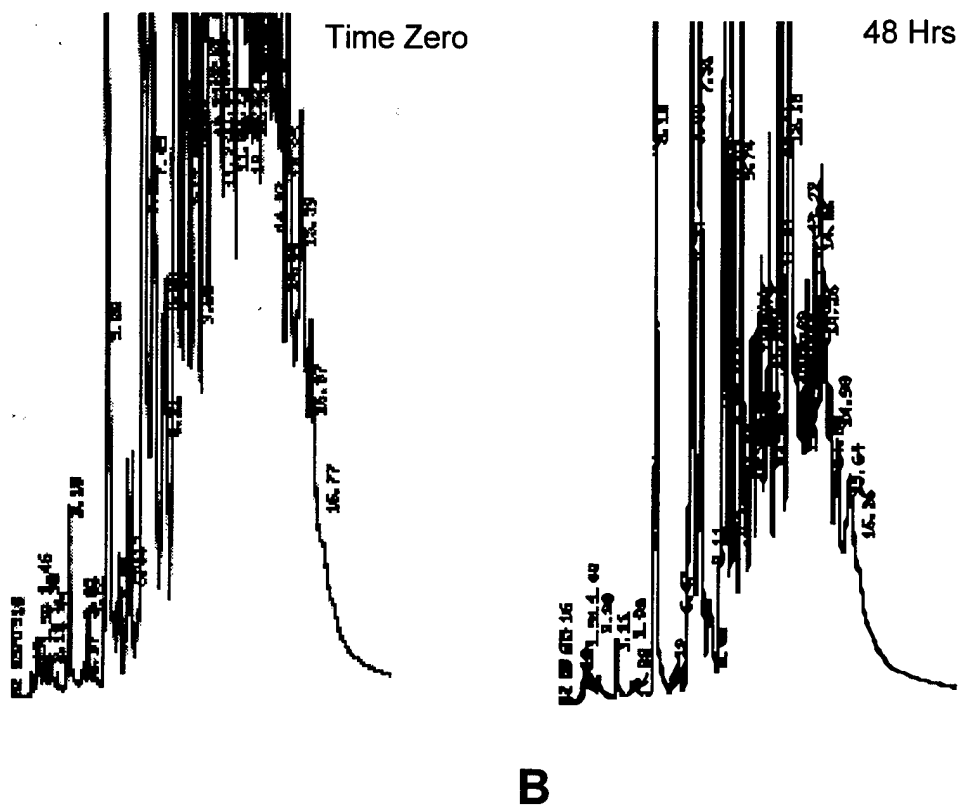
Figure 2. Chromatograms from a SAM dosed with 15 percent WSF, time zero (A) and 48 hours post dosing (B).

Treatment 4, SAM JP-8-1 Benzene Degradation

A   Time (Hours)
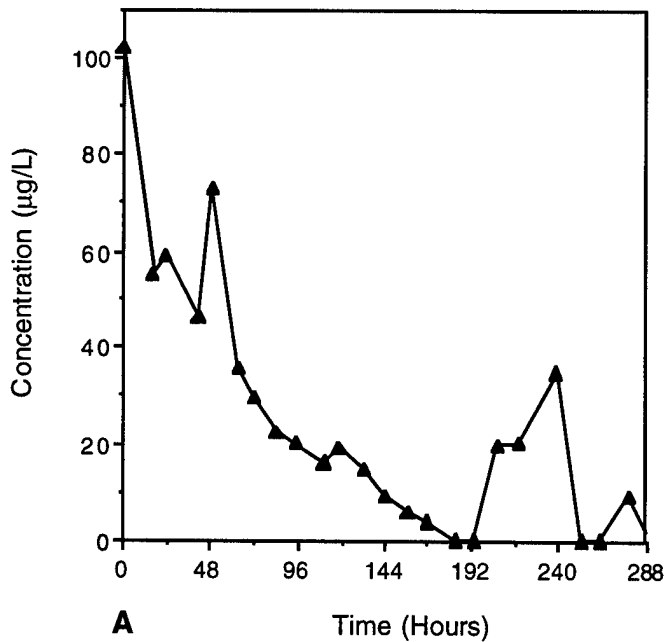
Treatment 4, SAM JP-8-1 SAM Toluene Degradation

B   Time (Hours)
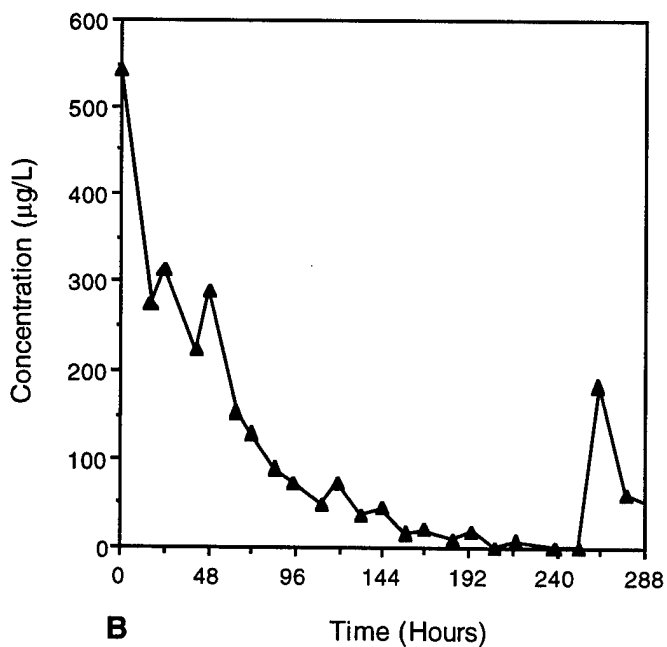
Figure 3. The concentrations of benzene (a) and toluene (b) in the SAM-1 experiment. The samples are taken from a SAM dosed with 15 percent WSF.

Summary of Univariate Results

AlgalPopulationDynamics: The population dynamics of the algae are presented in Fig, 4 and 5 for the JP-8-1 and JP-8-2 microcosm experiments respectively. In Fig. 5, day 63 is noted by a vertical line for ease of comparison to the normal period of a SAM experiment. Note that the x and y axes are of different scales in the figures to best represent each experiment.

In Fig. 4, the zero percent and 1 percent WSF treatments (Fig. 4a and 4b)appear to be similar. In both treatment groups the algae sharply declined, then increased again after day 21. Fluctuations were then observed until the end of the experiment. Stigeoclonium was a major part of both treatments during the later 2/3 of the experiment. Treatments 3 and 4 were similar in that the initial density of algal cells is not as high compared to the first two treatment groups. Although not as high a peak density was reached, the algae persist at a higher density for a longer time in Treatment 4. This persistent increase was statistically significant (IND method). Stigeoclonium is again an important part of the algal assembly during the latter segments of the experiment.

Fig. 5 summarizes the JP-8-2 experiment. In the first two treatment groups were again similar (Fig. 5a and 5b). The densities do not reach the same initial densities as in the JP-8-1 experiment, yet both demonstrated the initial reduction in density around day 20. At day 21 both assemblages see a rapid increase in algal density due to Anabena, Stigeoclonium and Lyngbya. The densities then decline until only low numbers of cells are present. The 5 percent treatment (Fig. 5c) sees a delay in the Anabena, Stigeoclonium and Lyngbya bloom until day 42. After the bloom occurs the numbers of cells then decline. Although the JP-8 is toxic to algae, the highest treatment group (Fig. 5d) has the highest numbers of algae and the populations persist the longest. During the period from day 28 until day 84, Anabena and Scenedesmus were the principal types in the algal assemblage. The total algal densities were generally highest in treatment 4. As in the other treatment groups, the numbers of algae were low during the last stages of the experiment.

Diversity has often been used as a measure of the structural status of an ecological assemblage or community. Figure 6 presents the Shannon-Weaver diversity for the algal assemblage for both experiments. In each experiment there was an initial decline in diversity that apparently starts to level off at approximately day 42. After day 42 neither experiment demonstrated much change is diversity except for a fluctuation in the Treatment 1of JP-8-2 between day 91 and 105. As defined by the use of the IND, JP-8-1 showed only an occasional single event divergence in diversity from Treatment 1. In the JP-8-2 experiment Treatment 4 did initially have a higher diversity than that of Treatment one in the early stages. After day 35, no more divergences from the diversity range of Treatment 1 were noted.

Total daphnia (Fig.7) reflected the concentration-response effects in both experimental systems for during the first 42 days. In JP-8-1 the lag in daphnia growth for the first 28 days was clear and follows the order, from most to least growth of Treatments 1, 2, 3, and 4. Treatment 2 is actually lower than Treatment 1 on day 14, but is not significantly different thereafter. In the JP-8-2 experiment Treatments 3 and 4 are lower than Treatment 1 until day 21. Treatments 2 and 3 occasionally overshoot Treatment 1 and Treatment 4 is significantly lower during days 21-51. After day 51 no significant divergence from Treatment 1 is apparent. The pH measurements also demonstrated the divergence of Treatment 4 from Treatment 1 (Fig. 8). In JP-8-1 (Fig. 9a), both Treatments 3 and 4 exhibit an excursion outside of the IND during the first 28 days of the experiment. No consistent differences were observed for the rest of the 63 day experiment. JP-8-2 (Fig. 8b) only Treatment 4 diverged from the IND in having a higher pH, in the first 42 days of the experiment. However, from days 98 until day 126 both Treatments 3 and 4 had a lower pH than within the range of Treatment 1.
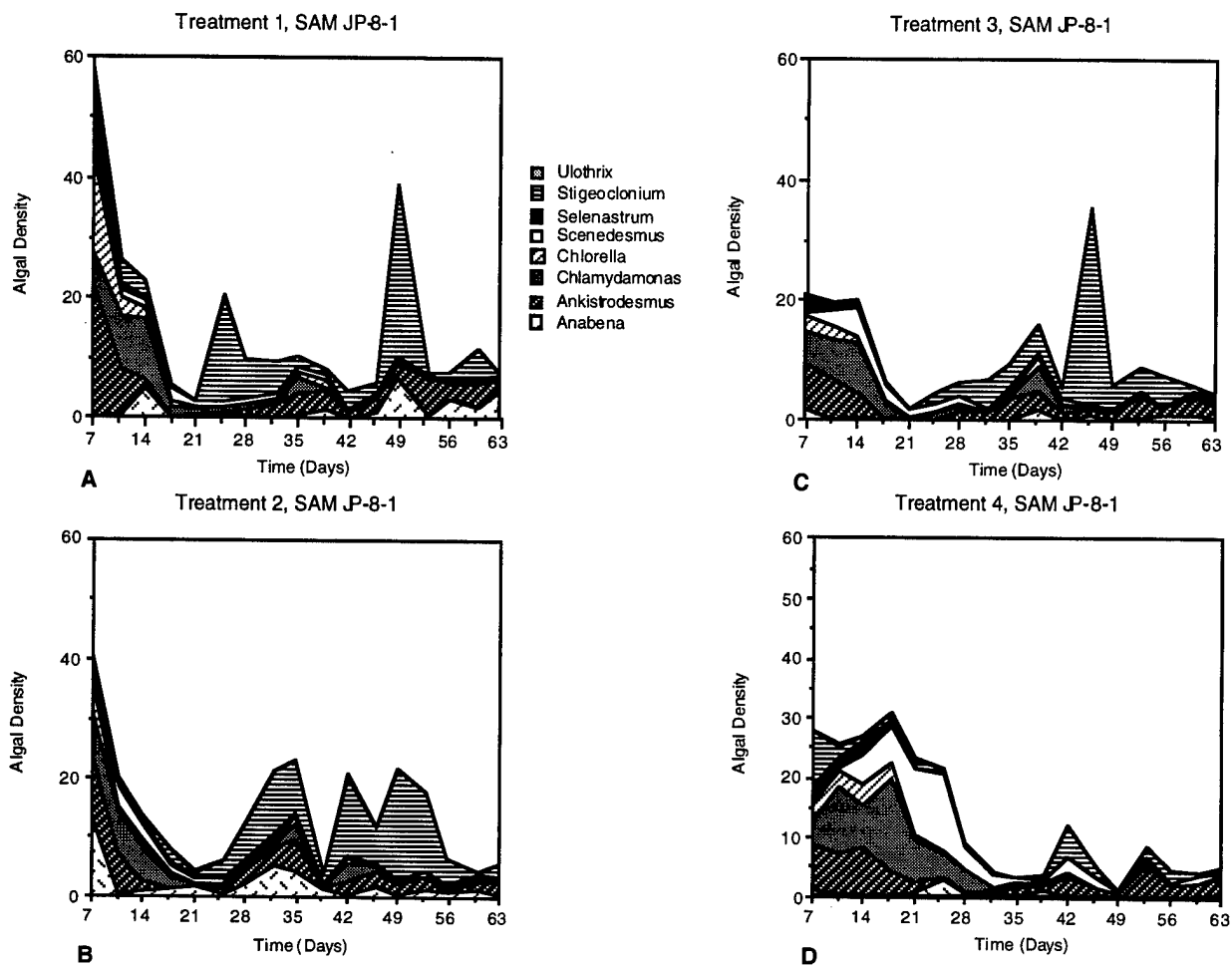
Figure 4. Algae population dynamics JP-8-1 within the four treatments.
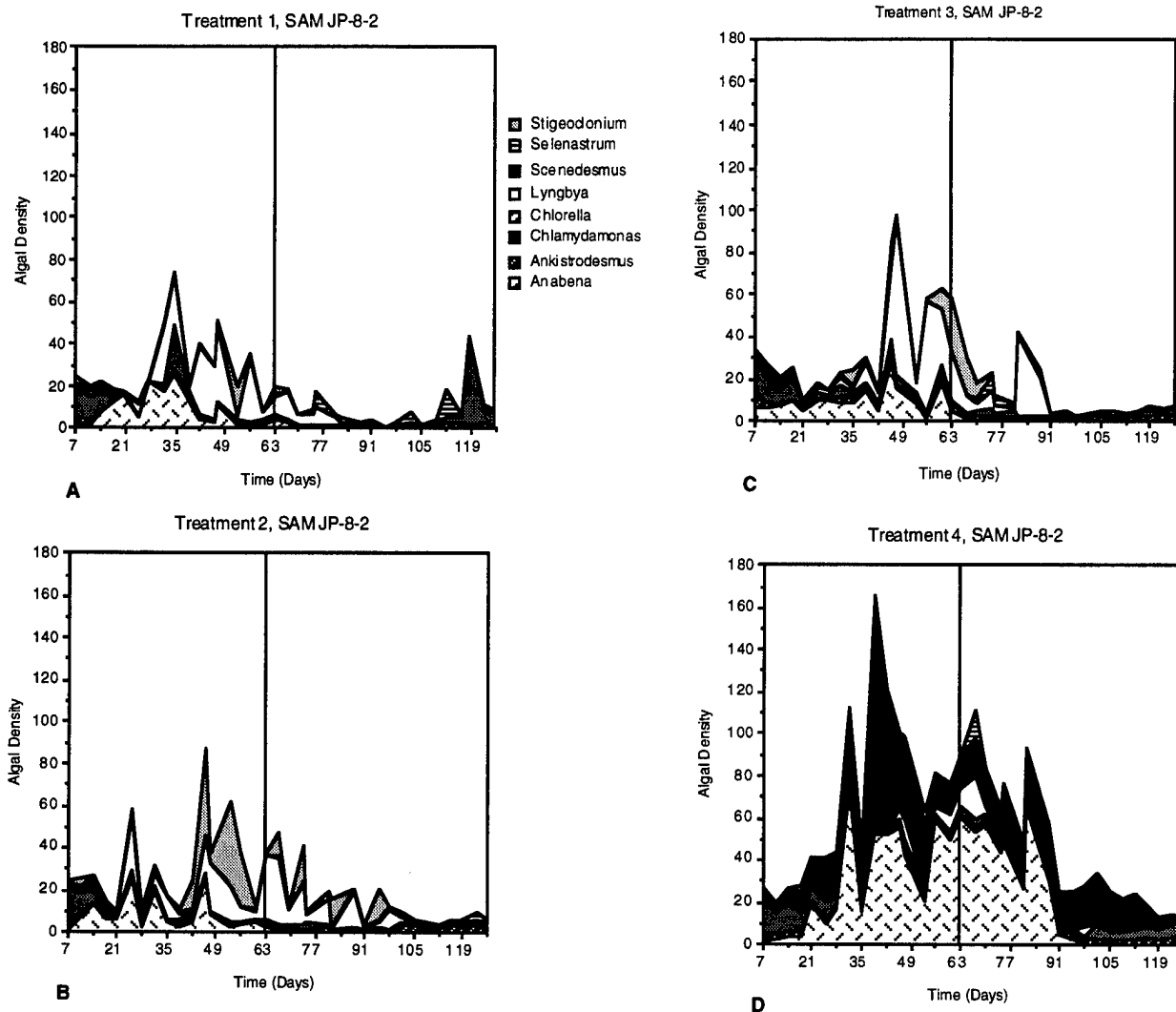
Figure 5. Algae population dynamics for JP-8-2 among the four treatments.
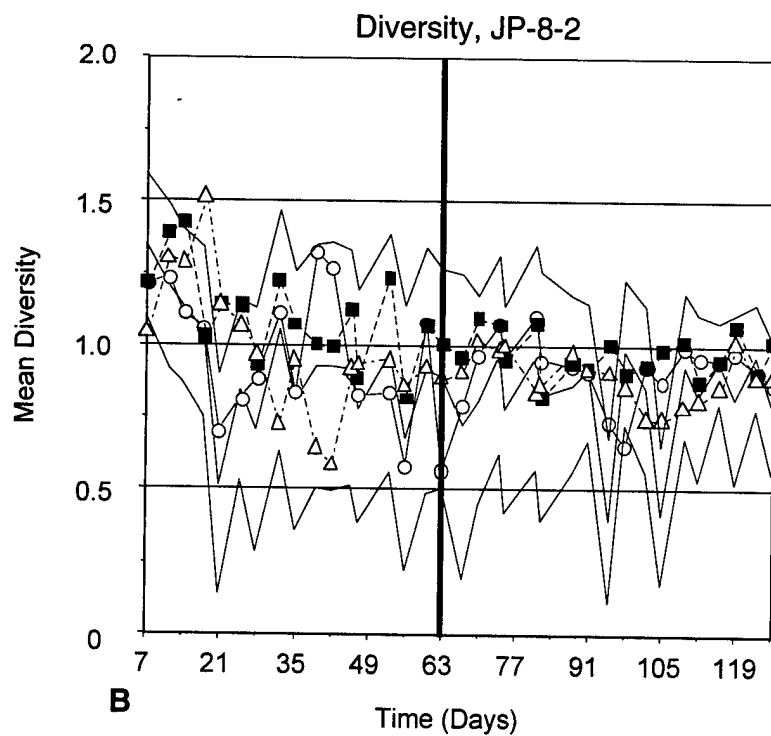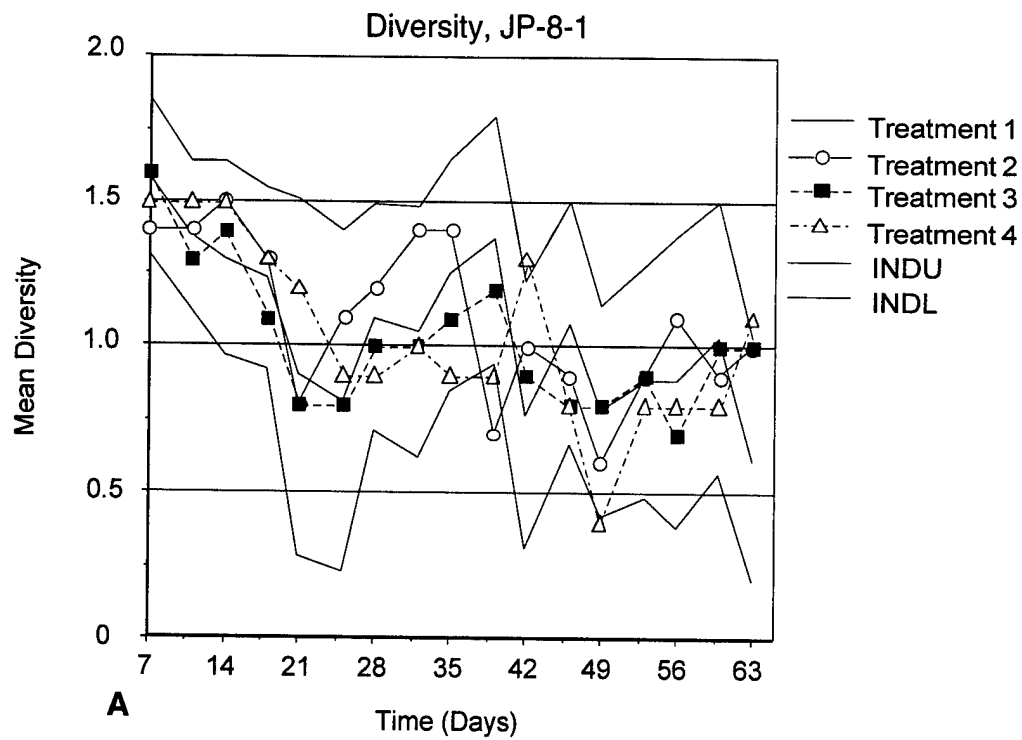
Figure 6. Algal diversity
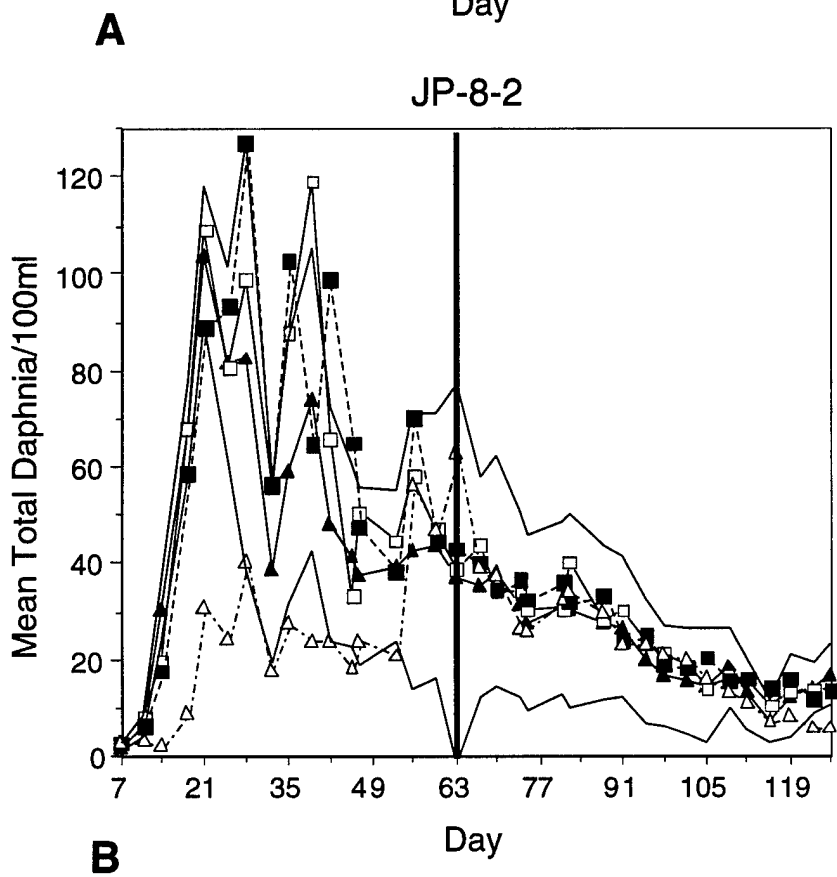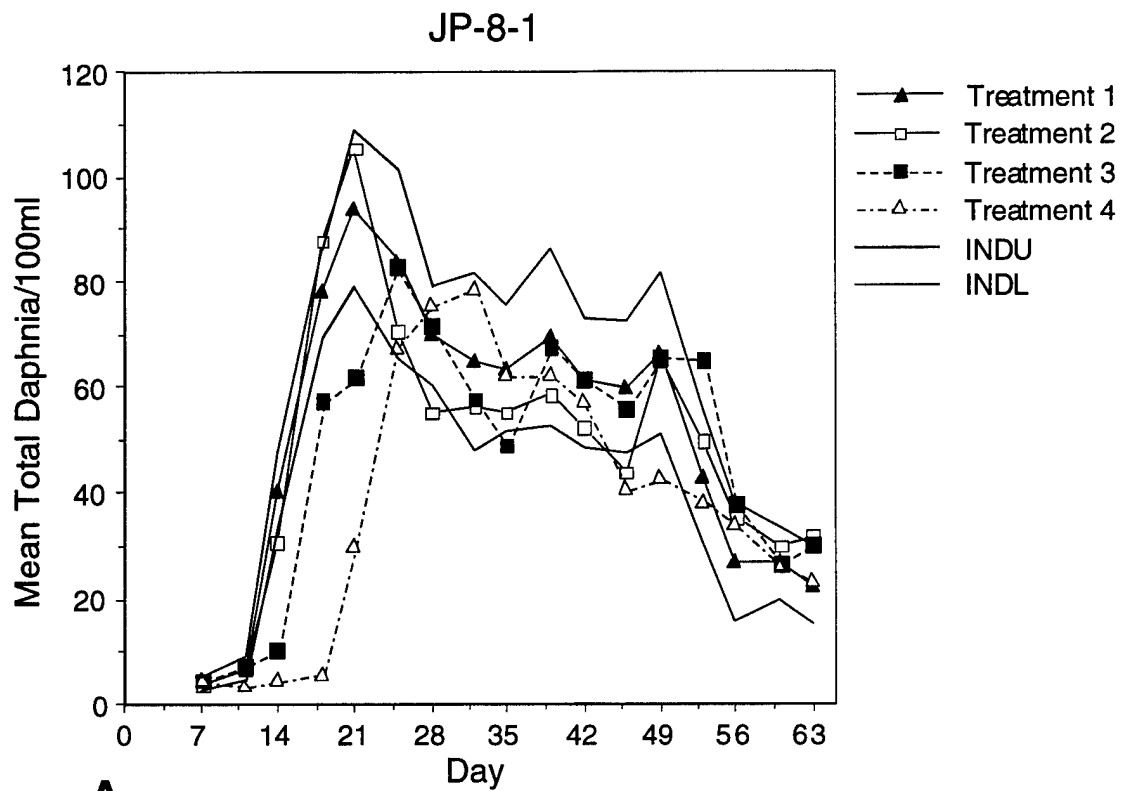
Figure 7. Total Daphnia JP-8-1 and JP-8-2
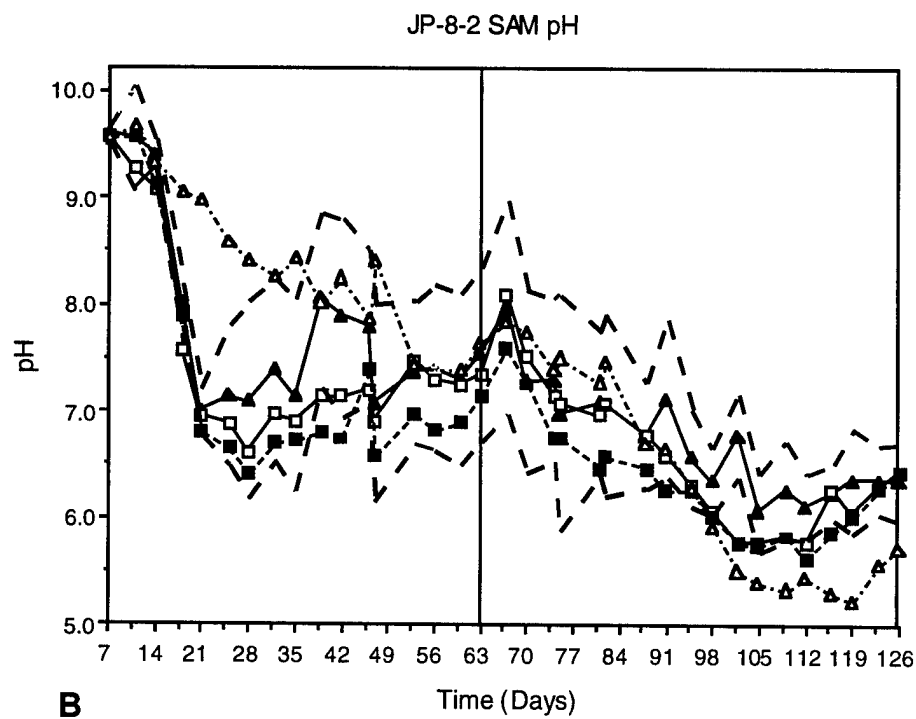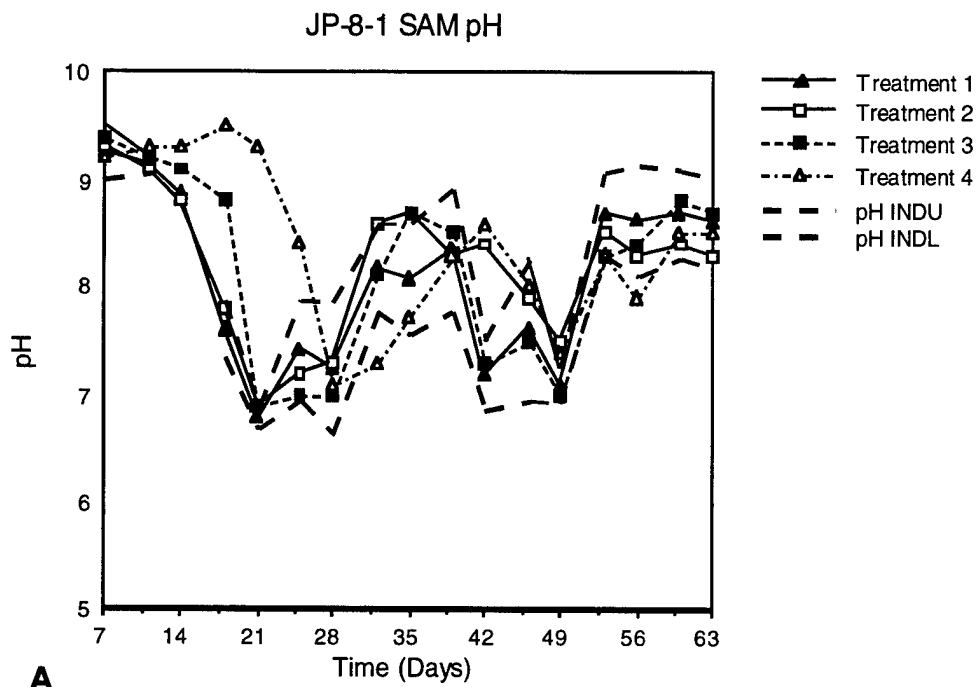
Figure 8. Comparison of pH for the JP-8-1 and JP-8-2 experiments.

Summary of Multivariate Results.

The statistical significance of the three clustering methods used are presented in Figure 8. The NCAA was clustered using the best 10 variables with 20 retries. In both experiments, clustering was associated with treatment during the majority of the experiment. It is also apparent that the three methods were not equivalent. On certain sampling dates the NCAA was better at detecting clusters than the other methods. At other times cosine or Euclidean clustering was better. At the end of the 63 day experiment JP-8-1 (Figure 9a) none of the methods were able to detect a clustering associated with treatment group. However, the longer experiment, JP-8-2 (Figure 9b) demonstrates that the effects of the JP-8 can persist well beyond 63 days.

A direct comparison of the NCAA results for the two experiments demonstrate that comparable results were obtained during the first 63 days of the experiment. At times no significant association was detectable, but the association reappeared. The cluster quality in both experiments peaked between days 14 and 21, followed by a general decline and then stabilization until the end of the experiment.

A difficulty in the evaluation of the association data is that each date is clustered separately and there is no correction of the multitude of statistical tests conducted. In order to ensure that the statistically significance was not spurious, we also incorporated cluster quality into our analysis. Two cluster quality values were used, 0.5 as in the published evaluations of other fuels, and 0.6 as used in a previous evaluation of the JP-8 data (Matthews et al 1996).

Table 3 presents the variables that were important in determining the clusters for each sampling date during the first 63 days for each experiment. In order to be considered important the contribution to the cluster quality has to be better than the average cluster quality for that sampling date. A pattern of green algae and daphnia being important in the early stages of the experiment followed by the appearance of ostracods and bluegreens during the latter stages was common to both experiments. However, the correspondence of variables being important in clustering was never exact on the same sampling date and on some days no variables were in common.

Table 4 presents a comparison of the ranks of the important variables for both experiments for the fist 63 days. The memberships of the four top ranks are identical except for P/R ratio being included. Although the membership overlaps, only pH has the same ranking in both experiments.

Further comparisons can be made by examining the JP-8-2 experiment for days 64-126 and as an complete experiment (Table 5). Days 64-126 exhibited a different ranking of important variables compared to the earlier phases of the experiment. Of the top four rankings only two variables are in common, Small Daphnia and Chlamydomonas. This change in rankings is reflective of the change in the microcosm becoming a detritally based system. The correspondence of variables of the first four ranks over the entire experiment to the first 63 days with the entire experiment is high with four of the five variables in common.

Table 6 portrays the membership of each treatment group into each cluster for both experiments. In the JP-8-1 experiment the most common pattern was that two clusters were best. Typically treatments 1 and 2 comprised one cluster and 3 and 4 the other. Treatments 1, 2 and 4 each comprised its own cluster at some point in the experiment. In the JP-8-2 experiment, two, three or four clusters were the best in almost equal numbers. Treatments 1,2,3 and 4 all comprised separate clusters at some time during the experiment. Treatment 4 stood alone the most often of any of the treatment groups. The patterns are quite diverse. On day 47, treatments 1 and 4 comprise a cluster while the intermediate treatments are in distinct clusters. ON other dates the clusters are as expected, treatments 1 and 2 forming one cluster and 3 and 4 the other (days 21, 82, 88).
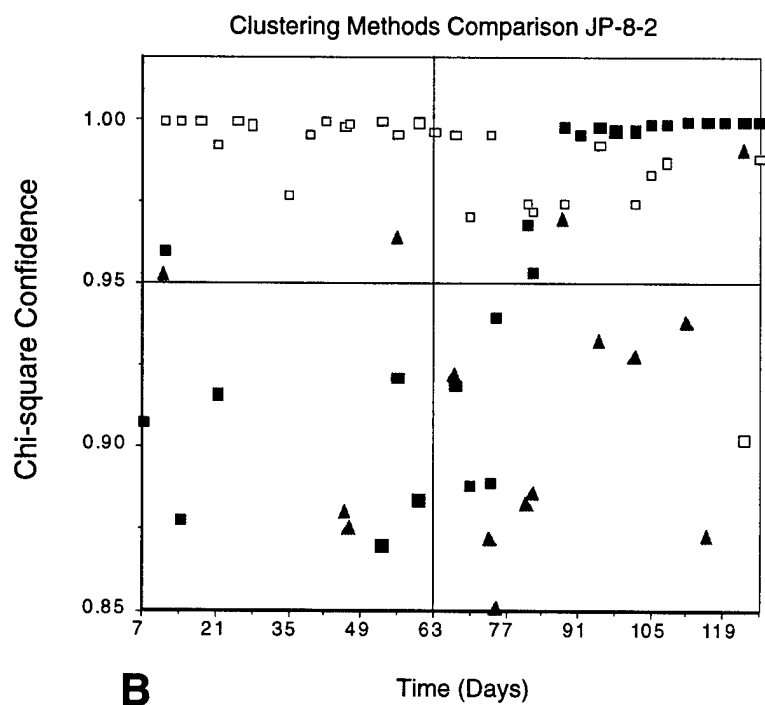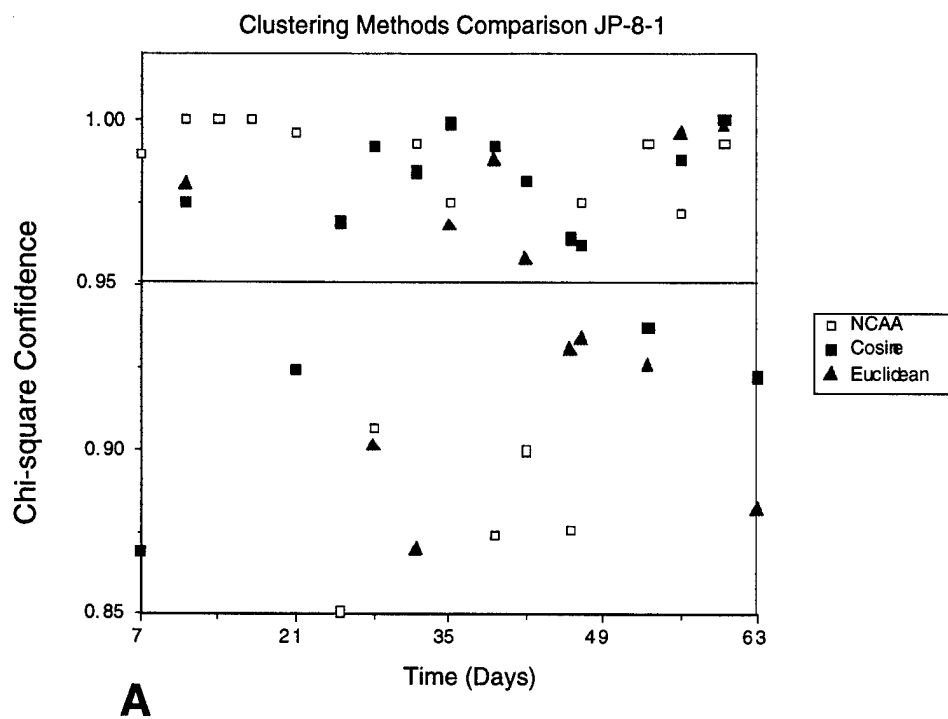
Figure 9. Clustering for both microcosm experiments using NCAA, Euclidean and cosine techniques.

Toxicant	Organism	IC50/EC50 percent WSF
JP-8	Ankistrodesmus	29.8
	Chlamydomonas	65.5
	Selenastrum	15.3
	Ankistrodesmus	14.8
	Chlamydomonas	24.2
	Ankistrodesmus	32.5
	Selenastrum	62
	D. magna	10.8
	D. magna	12.5
	D. pulex	22.8
Crude Oil	Selenastrum	16.0
	D. magna	9.0

Table 1. Comparison of JP-8 to the toxicity of crude oil. In each experiment the toxicant is the water soluble fraction of each of the materials listed. WSF=Water Soluble Fraction. T82=microcosm media. LW=Lake Water. The algal tests were conducted following ASTM E 1218-90. Daphnid toxicity tests were conducted following the protocols of ASTM E 729-88 and Biesinger (1987).

JP-8sam1RIFFLEdata	JP-8sam2RIFFLEdata	Correspondence
Day		
7	Scen(1)-Ankist(2)-Chlor(2)-Sele(3)-Tetra(4)	Ostr(1)-OD(2)-SDaph(3)-Chlam(4)-Chlor(4)	1
11	LDaph-pH	PR(1)-OD(2)-pH(2)-MDaph(3)-SDaph(4)	1
14	SDaph-LDaph-pH	SDaph(1)-LDaph(2)-PR(2)-Ankist(3)-Phil(4)	2
17	SDaph-pH-Ankist	Chlor(1)-Scen(1)-SDaph(2)-Tetra(3) Ankist(4)	1
21	Scen(1)-pH(1)-Ankist(2)-SDaph(2)-OD(2)	Ankist-SDaph-pH-MDaph-Chlam	3
28	Scen-Chlam-Stig-Ulot-Lyngbia	Chlor-Ankist-MDaph-Chlam-PR	0
32	pH-Ankist-Phil-Ulot-OD	PR(1)-Ankist(2)-Scen(2)-SDaph(3)-pH(4)	2
35	Ankis(1)-pH(1)-Scen(2)-Stig(2)-LDaph(3)	SDaph(1)-PR(2)-pH(3)-MDaph(4)-OD(4)	0
42	pH(1)-MDaph(2)-Epph(3)-Ankist(4)-Stig(4)	SDaph(1)-pH(2)-MDaph(3)-Epph(4)-OD(4)	2
46	Anab(1)-Ankist(2)-OD(3)-Chlam(4)-Epph(4)	Epph(1)-Scen(2)-SDaph(3)-Ankist(4)Chlam(4)	2
47	Epph(1)-Chlor(2)-Ankist(3)-Sele(3)-pH(4)	pH-PR-Chlam-Phil-OD	1
53	Chlam(1)-Lyng(1)-Scen(2)-Ankist(3)-PR(3)	Ostr(1)-Phil(2)-pH(3)-Chlam(4)-Anab(4)	1
56	Chlor-SDaph-Scen-MDaph-LDaph	Stig(1)-Sele(2)-PR(3)-Scen(4)-Anab(4)	1
60	Ostr-Phil-MDaph-Chlor-Epph	Chlam-Lyng-Ankist-pH-SDaph	0
63	SDaph(1)-Ankist(2)-Epph(3)-MDaph(4)-pH(4)	PR-Ankist-Lyngbia-pH-Anab	2

Table 2. Comparison of Riffle Results for JP-8-1 and JP-8-2 for the First 63 Days. The important variables for each sampling date are presented and compared. Although the experiments are an attempt at the replication of a multispecies toxicity test, the correspondence of important variables is not particularly high. On one date (day 21) three of the variables are common to both experiments. Note that there is no apparent pattern. Both the early and late stages of the experiments have a low number of corresponding variables.

Rank	JP-8-1	JP-8-2
1	Ankistrodesmus	SmalDaphnia
2	pH	pH
3	SmalDaphnia	Chlamydomonas-PR
4	Chlamydomonas	Ankistrodesmus
5	Chlorella-Scenedesmus-Ephippia	Anabaena-MediunDaphnia-Optical Density
6	Stigeoclonium-MediurBaphnia-LgDaphnia	Scenedesmus-Lyngbia-Ephippia
7	Selenastrum-PR-OpticaDensity	Chlorella-Philodina
8	Ulothrix-Lyngbia-Philodina	Ostracods
9	Anabaena-Ostracods-Tetrahymena	Selenastrum-Stigeoclonium Tetrahymena-LargeDaphnia

Table 3. Comparison of the ranks of important variables. These are the results of Riffle when the clustering is based on the 5 best variables. The top four ranked variables are shared in each experiment although the order of the top four is different except for pH.

Days 64-126	Days 1-126
OpticalDensity	SmDaphnia
MedDaphnia-Ostracods	pH
Selenastrum-SmDaphnia	Chlamydomonas
Chlamydomonas	PR
PR	MedDaphnia
Philodina	Ankistrodesmus-Ostracods
Ankistrodesmus-Lyngbia-Ephippia-OpticaDensity	OpticalDensity
Tetrahymena	Selenastrum
Chlorella	Lyngbia-Ephippia-Philodina
Stigeoclonium-Anabaena Scenedesmus-Anabaena	Tetrahymena
	Stigeoclonium-Chlorella-LgDaphnia

Table 4. Rank order of the JP-8-2 Experiment.

Table 5. Composition of the Clusters obtained by NCAA for the JP-8-1 and JP-8-2 experiments. The treatment groups are 1, 2, 3, 4 for the 0, 1, 5, and 15 percent WSF treatments respectively. Cluster 1 is always the cluster comprised primarily of treatment 1and so forth. Note that often only 2 or three clusters are present in the data.

JP-8-1

Day	Cluster 1	Cluster 2	Cluster 3	Cluster 4
7	1	1,2,3	3,4	
11	1,3	2	1,3	4
14	1,2	3,4		
17	1,2	3,4		
21	1,2	3,4		
25	Not significant			
28	Do over			
32	1,2,3	3,4		
35	1,2	3,4		
39	Not significant			
42	Not significant			
46	Not significant			

47	1,2,3	4		
53	1,2	3,4		
56	1, 2	2,3	1,4	1,2,3,4
60	1,2,3	4		
63	Not significant			

JP-8-2

7	Not significant			
11	1	2	3	4
14	1	2,3	2,3	4
18	1,2	2,3	3	4
21	1,2	3,4		
25	1,2	2,3	4	
28	1	1,4	2,3	
32	Not significant			
35	1,2	3	1,4	
39	1,4	2,3		
42	1,2	3	4	
46	Not significant			
46	1,2	1,3	4	
47	1,4	2	3	
53	1,2	2,3	3,4	
56	1,2	3,4		
60	1,2	3,4		
63	1,3	2	2,3	4
67	1,4	2,3		
70	1,2	2,3	3,4	
74	1,2	3,4		
75	Not significant			
82	1,2	3,4		
88	1,2	3,4		
91	Not significant			
95	1,2	3,4		
98	Not significant			
102	1,2,3	2,3,4		
105	1,3	2,3	2,4	4
108	1,2	1,3	3	2,1
112	Not significant			
116	1,2	1,2	3	4
119	Not significant			
123	Not significant			
126	1,2	1,2	2,3	4

Discussion

These experiments confirmed the persistence of concentration related clusters during the course of each experiment. Significant clustering would occur, then perhaps a period were treatment effects could not be detected, followed by a re-establishment of the clustering related to treatment. This pattern was the most pervasive and common throughout this series of experiments even though the toxicant is no longer detectable within the water column. These results are comparable to previously published (Landis et al 1993a, 1993b, Matthews, Landis, Matthews 1996) microcosm experiments.

Ecosystem Dynamics and the Importance of Non-equilibrium Conditions

The return of a system to its pre-existing state, structurally, metabolically and dynamically, is a classical definition of recovery. The property that confers upon a system the ability to recover is stability. It has often been assumed the stability is a property of persistent ecological systems. It has even been suggested that the examination of stability and the measurements of resilience and recovery are the most appropriate attributes to be studied in multispecies toxicity tests. Even in situations where an equilibrium does not occur it is assumed that given more time that replicate systems will converge toward an equilibrium condition (Rosenzweig and Buikema 1994). As comforting as an assumption of ecological stability may be, there is an increasing amount of data that indicate that stable systems may be the exception.

The return of a system to its pre-existing state, structurally, metabolically and dynamically, is a classical definition of recovery. In regard to populations, Connell and Sousa (1983) examined a great deal of the literature on population dynamics and found stability as return to original conditions extremely rare. Andrews (1991) in a study of tropical lizards found that the population dynamics are unstable. Hypothesized causes are the rapid population turnover and the complexity of a food web. Over the last ten years it has in fact been found that many populations exhibit chaotic dynamics (Schaffer and Kot, 1986, Hastings et al 1993). Although density dependent regulation is operating, the populations are characterized by large unpredictable fluctuations that are inherently unpredictable. In fact there is ample theory that predicts the inherent instability of large dynamic and connected systems (Gardner and Ashby 1970, May 1972). Given the unpredictability of outcomes in a variety of theoretical (Yodzis 1988) and experimental (Thompson et al 1991)cases, an assumption about the reality of stability and the reliance upon the measurement of recovery seems improper.

Recovery as a value, not ecological reality

Although we were able to detect patterns that are distinctive of treatment groups during the entirety of both experiments, the densities of organisms and the chemical parameters are not substantially different. Among most of the variables no significant difference was observed. Typically, when this state has occurred the claim of recovery is made. In one instance the structure of a freshwater macrophyte community was altered by the application of Atrazine, yet because the biomass was equivalent the claim of recovery was made (Solomon et al 1995). In another instance data were eliminated that countered the claim of recovery because it did not have the resistant life stages of other macrobenthos and had to re-colonize the artificial stream systems. Eliminating the variable allowed a claim of recovery to be made.

In both instances recovery could be claimed if only one aspect of the community was observed or data that did not conform to this interpretation was eliminated.

Conclusions

1) Effects are persistent in these model ecological systems long after the demise of the toxicant. In the case of the 126 day experiment clusters that matched treatment groups were identifiable until the end of the experiment.

2) Patterns of impacts are detectable at concentrations 15 times lower than the experimentally derived single species EC_{50}.

3) The replicate experiments are not replicable in the specific, but the broad pattern of the disruption of algal, herbivore dynamics followed by more subtle effects are consistently repeated. These impacts are similar to those observed in the Jet-A and JP-4 experiments previously described.

4) The durability of the indirect effects and therefore the information about historical events appears to be a consistent feature of these microcosm systems. At time the ability of our

techniques to detect patterns is diminished, but the re-emergence of patterns related to treatment is a consistent property of these experimental systems. Although the replicates may be constrained in ecosystem space by nutrient limitations, the identity of the treatment groups persist.

5) The critical features of the community conditioning hypothesis: persistence of information within an ecological systems, the reappearance of patterns and therefore the non-equilibrium dynamics have again been confirmed.

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Section 3 - Outdoor Mesocosms

Introduction

We developed an outdoor mesocosm system that incorporates detrital conditioning to test the hypothesis that microbiota play a critical role in altering the community response to hydrocarbon toxicants. The mesocosms were constructed using 568 L tanks, arranged in 6 units of 4 tanks, with each unit equidistant from a central conditioning tank. During pre-treatment, the mesocosms and conditioning tank were filled with nutrient-amended well-water (500 (g-N/L and 20 (g-P/L), artificial sediment (silica sand, ground chitin, and cellulose powder), leaf packs containing dried maple leaves, elodea fragments, and unglazed tiles for periphyton growth. Water circulation was maintained at the rate of 24 exchanges per day. After four weeks, invertebrates from local ponds were added to the conditioning tank. Leaf packs were added to the conditioning tank and mesocosms every two weeks; eight week old packs were discarded after returning invertebrates to the conditioning tank. On a weekly basis, 25% of the sediments, leaf packs, tiles, and elodea from each mesocosm were transferred to another mesocosm; the conditioning tank walls and tiles were scraped; and the water quality was monitored. Circulation was discontinued one week prior to dosing. On April 12, 1996, the mesocosms were dosed with 0-, 19-, 57-, and 97 mL of neat JP-8 jet fuel, resulting in the following concentrations of JP-8:

TREATMENT GROUP	mL JP-8	CONCENTRATION
Group 1	0.0 mL	0.0 mL JP-8/L
Group 2	19.0 mL	0.05 mL JP-8/L
Group 3	57.0 mL	0.15 mL JP-8/L
Group 4	95.0 mL	0.25 mL JP-8/L

The dose was chosen to provide hydrocarbon concentrations similar to those used in earlier laboratory-based Standardized Aquatic Microcosms (SAM) and Mixed Flask Culture Microcosms (MFC) tests. The post-exposure experiment duration was 208 days.

Methods

Constructing the Outdoor Mesocosms

This section describes the procedure for the construction of an outdoor mesocosm experiment, including the design, materials, set-up, and the conditioning of mesocosm communities. Figures 1-4 show the finished conditioning tank and mesocosms at the IETC research site.

Materials

1)_340 L Mesocosms (568 L capacity), fiberglass, oval, (63.5 cm x 147.3 cm or 150 gal oval fiberglass cattle troughs, 58 in x 25 in).
2)_12,000 L Conditioning Tank, with 30 mil PVC liner, (116.84 cm x 365.76 cm stainless steel tank, round, 15 gauge, 11771.35 L or 3110 gal capacity).
3)_Pump, TEEL Stainless steel, self-priming, centrifugal with TEFC motor, Teflon seals. 3/4 HP.
4)_1.905 cm PVC SCH-40 irrigation pipe (3/4 in), elbows, couplings, butterfly valves, and unions.
5)_3.81 cm PVC SCH-40 irrigation pipe (1 1/2 in), elbows, couplings, butterfly valves, and unions.
6)_Plastic sheeting, 2 rolls, clear, 12 mil, (15.24 cm x 269.24 cm or 6 ft x 106 ft)
7)_Sand, (15.294 m3 or 20 yd3)
8)_Tarpaulins, polytuf, heavy duty (168 cm x 584.64 cm or 14 ft x 18 ft)
9)_Water source, analytically examined and characterized.

Site Preparation and Conditioning Tank Installation

A 15.5 m x 15.5 m area was cleared of all vegetation, large rocks and other debris. The excavated material from the center area was used to form a 46 cm high berm around the

entire15.5 m x 15.5 m area. The center of the site was excavated to a level depth of 1.22 m (4 ft) and a radius of 3.96 m (13 ft). Bricks (15.24 cm x 2.54 cm or 6 in x 1 in) were evenly spaced around the circumference of the excavation to act as supports for the tank wall. The 12,000 L stainless steel tank was assembled and placed on the bricks so that it was stable. Sand (1.6 m3 or 2.1 yd3) was spread evenly in the bottom of the tank, 15.2 in (6 in) deep, to form a cushion for the tank liner. The tank liner was placed in the tank according to manufacturer's instructions and secured to the top of the tank. Additional sand was spread around the outside of the tank and tamped down to provide thermal insulation and support for the tank walls. A mixture of sand (1.15 m3 or 1.5 yd3), 400 g of ground chitin, and 400 g of cellulose powder was placed in the bottom of the tank to serve as an initial substrate (ASTM-E 1366-91). The tank was filled with source water so that the level of water was 0.15 m below the top of the tank. The area around the conditioning tank was graded and leveled. Overlapping sheets of heavy duty tarpaulins were placed on the graded area to cover the ground from the sides of the conditioning tank to the edge of the berm. The tarpaulin area was covered with 7 m3 (9.2 yd3) of sand up to the edge of the berm to secure the tarpaulin and cushion the mesocosms.

Mesocosm Placement and Set-up

Six groups of four 568 L capacity mesocosms were arranged equidistant from the center of the central conditioning tank. The mesocosms were placed in a 2 x 2 matrix and spaced approximately 0.5 m apart. Dry sand (0.048 m3 or 0.063 yd3) was placed in the bottom of each mesocosm, with care taken not to block the intake opening mid-way along the side of the mesocosm and near the bottom. The sand covered the bottom to a depth of 0.03 m. Ground chitin and cellulose powder (63.3g each) was mixed with the sand in each mesocosm.

Flow-through System Design

Plumbing and Installation

A 3.81 cm PVC pipe was plumbed from the conditioning tank to the inlet of the pump and from the outlet to a six-port manifold located directly above the conditioning tank. From each port on the manifold, a 3.81 cm PVC pipe was plumbed to each four-port manifold located in the center of each 2 x 2 group of four mesocosms. A butterfly valve was located inline to the four-port manifold so that each group of four could be isolated from the flow-through system. From each port on the four-port manifold, a union fitted with an inline restrictor disk (1 cm ID opening) was plumbed into a1.905 cm PVC pipe that connected to the mesocosm intake. The mesocosm intake was a 3.81 cm threaded opening located on the side of the mesocosm, approximately 3.8 cm from the bottom, and midway down the mesocosm length. Each mesocosm was fitted with a 3.8 cm overflow pipe located 0.15 m from the top of each mesocosm. Each overflow pipe was covered with Mesh #18 (1 mm) to prevent loss of macroinvertebrates from the mesocosms. The overflow pipes from each group of four mesocosms were plumbed into a 3.81 cm PVC pipe that extended back to the edge of the conditioning tank.

Water Circulation and Flow

Water was pumped from the conditioning tank to the six-port manifold located directly above the conditioning tank. Water was routed from each port on the manifold to one of the four-port manifolds located in the center of each group of four mesocosms. Water was routed from each port on the four-port manifold through an inline restrictor disc to reduce the flow to 0.11 L/sec before it entered the intake port of the mesocosm. The water was split into two opposite streams as it entered the mesocosm and encountered the opposite wall. Each stream circulated the water in each half of the mesocosm in a slow moving, circular pattern. Water entered the mesocosm through a 3.81 cm threaded opening approximately 3.8 cm from the bottom, at the midpoint of the mesocosm. At capacity the mesocosm water levels were maintained to within 0.15 m of the mesocosm top by the outflow pipe that routed the overflow from the group of four mesocosms back by gravity to the conditioning tank. Mesocosm volumes were replenished at a rate of one complete exchange per hour. Supplemental water from the designated water source was supplemented to the conditioning tank to compensate for losses due to evaporation and

monitoring procedures.

Criteria used to minimize water flow and spatial variation

Pipe lengths and sizes were kept identical within each section of the distribution network. Water flow was regulated into each mesocosm using inline restrictor disks to produce twenty-four changes of water per day per mesocosm. Mesocosm overflow outlet screens were cleaned regularly to ensure water levels remained consistent. The position of the six groups of four mesocosms was equidistant from conditioning tank. The position of the three groups of four mesocosms on one half of the site were positioned identically with reference to the three groups of four mesocosms on the other half of the site, regardless of the orientation of the center line. The position of each mesocosm in each group of four was identically arranged in a 2 x 2 matrix, with the long side parallel to the east-west axis.

Canopy Construction

Canopies for the conditioning tank and each of the individual mesocosms were constructed to prevent precipitation from entering the systems and to exclude airborne debris, insects, and contaminants. PVC pipe (1.905 cm), elbows, and tees were used to make the mesocosm canopy frame. The frame was built in the shape of a right triangle measuring 163 cm in length and 126 cm wide. Cut sections of 12 mil clear plastic measuring 305 cm x 183 cm were secured on two sides of the triangular framework with heavy duty clear tape. The base of the frame was fitted with four short 1.905 cm PVC pipes that were adapted to be screwed directly onto the top of the mesocosm rim. The canopy was slightly larger than the outside edge of the mesocosms, allowing full protection, but maximum accessibility. The open ends of the mesocosm canopies were covered with plastic screen mesh to retain emergent species and exclude insect pests. On completion of flow-through system and canopy construction, sand was used to berm around the individual mesocosms and cover the 1.905 cm PVC pipes leading into the mesocosm intake ports. The sand provided protection and thermal insulation for the mesocosms and piping.

Establishment and Conditioning Phase

The conditioning tank and mesocosm flow-through system were circulated for three months prior to beginning the experiment.

Month 1

Water and sediments were collected from local freshwater streams, creeks, and lakes and inoculated into the conditioning tank and the individual mesocosms. Water and sediments were composited and mixed into a slurry. Two liters were added to the conditioning tank and 300 mL to each mesocosm. Eight labeled, unglazed tiles (15.24 x 15.24 cm) were suspended in each mesocosm to lay parallel to the sediment and at a depth of 0.2 m.
3)Ninety-six tiles were suspended similarly in the conditioning tank. Four labeled macrophyte packs containing 50 g (wet weight) of Elodea were added to each mesocosm and twelve packs were added to the conditioning tank. Every two weeks, four labeled leaf packs were added to each mesocosm and twelve packs were added to the conditioning tank. Each leaf packs contained 10 g of maple leaves (Acer sp.), air-dried for six months, with stems removed.

Month 2

Water and sediment were collected again from local freshwater streams, creeks, and lakes. One liter of sediment slurry was inoculated into each mesocosms and 10 L was placed into the conditioning tank. Each mesocosm was inoculated with 5 cladocera (*Daphnia pulex*), 5 amphipods (*Hyalella azteca*), 3 snails, (*Ampularia cuprina*), and 20 ciliates *(Paramecium bursaria)*. The conditioning tank was inoculated with 30 cladocera (*D. pulex*), 30 amphipods, (*H. azteca*), 20 snails, (*A. cuprina*), and 250 ciliates, (*P. bursaria*). Each week 2 L sediment, four leaf packs, four Elodea packs, and two unglazed tiles were transferred from one mesocosm to the next mesocosm in increasing numerical order. Each week four of the tiles in

each mesocosm were scraped to remove periphyton; the alternate four tiles were scraped the following week. The scrapings were returned to the mesocosms. Each week water quality in the conditioning tank was analyzed to measure pH, alkalinity, conductivity, hardness, turbidity, dissolved oxygen, temperature, soluble reactive phosphate, and nitrate/nitrite.

Pre-exposure Period (21 days)

Water quality parameters (alkalinity, pH, conductivity, hardness, turbidity, dissolved oxygen, temperature, light intensity, nitrate/nitrite, soluble reactive phosphate, and total organic carbon) and volatile organics (purge & trap gas chromatography) were measured weekly. Biweekly biological samples were collected to measure:

> Elodea wet weight
> Elodea ash-free dry weight (pruned portion only)
> 8-week leaf pack macroinvertebrate taxonomic abundance
> 8-week leaf pack wet weight
> 2-week periphyton taxonomic abundance
> 2-week periphyton ash-free dry weight
> 2-week periphyton total solids
> 2-week periphyton total volatile solids
> 2-week periphyton chlorophyll concentration
> 2-week periphyton TTC respiration
> phytoplankton taxonomic abundance
> phytoplankton ash-free dry weight
> phytoplankton total solids
> phytoplankton total volatile solids
> phytoplankton chlorophyll concentration
> phytoplankton TTC respiration

The 8-week old leaf packs were removed from the mesocosms after counting and returning all invertebrates to the mesocosm. The periphyton samples were scraped from 4 tiles (alternate tiles every two weeks).

Mesocosm and System Modifications (1 Week Before Treatment)

The re-circulation system was disconnected and the outflow was redirected to the overflow containments. The water level in each mesocosm was marked. All cross-inoculations and reinoculations of sediments and biota was stopped. The mesocosms were randomly assigned to treatment groups.

Exposure Period (208 days)

On day zero of the exposure period the following volumes of neat JP-8 were added to each of the six mesocosms in a treatment group:

Group	mL JP-8	Concentration
#1	0.00 mL JP-8	0.00 mL JP-8/L
#2	19.0 mL JP-8	0.05 mL JP-8/L
#3	57.0 mL JP-8	0.15 mL JP-8/L
#4	95.0 mL JP-8	0.25 mL JP-8/L

The JP-8 used for dosing was collected immediately after refining and shipped in sealed containers to our laboratory. The containers were opened at the time of treatment. Class A graduated cylinders were used to dispense appropriate volumes to each mesocosm. The jet fuel was carefully poured onto the surface of the water to distribute the fuel evenly as a surficial slick.

Mesocosm Measurement Parameters

Same as Pre-exposure, weekly for first 60-days, then biweekly for a total exposure period of 208 days. Elodea measurements were discontinued after the first month because the plants did not thrive.

JP-8 Jet Fuel Analyses

Purge & Trap/Gas Chromatography (P&T/GC) was used to analyze the concentrations of selected hydrocarbons in the water column. Peak areas and retention times were used for hydrocarbon identification and quantification. The FID signal was reported as Log10 transformed Peak Area.

Supplements to Mesocosms (Weekly)

Conditioned source water from the conditioning tank was added as needed to maintain mesocosm water levels. Four new leaf packs were added to each mesocosm every two weeks. Nutrient concentrations were amended as needed to maintain minimum of 20 µg/L SRP and 500 µg/L NO3.

Results and Discussion

Periphyton and Leaf Pack Data
Data Set Description

The periphyton and leaf pack data are located in the compact disk provided with this report. The data are in ASCII format in the file pl.dat (all sampling days) and in subfiles for each sampling date (e.g., pl_004.dat). The CD also contains README files that describe the contents of each data set and SPSS command files for producing descriptive statistics and single-day data sets. The verified Mesa spreadsheet files are located on the same CD.

All data were verified using double-entry, followed by subtraction of the duplicate files. Non-zero results were checked against the laboratory bench sheets and entered correctly. The corrected file was reverified by randomly selecting one row from each experiment day and checking all columns against the bench sheet entries. Any computational errors were corrected and the data were plotted to review outliers. Outliers were reverified against benchsheet entries and decisions concerning rejection or acceptance are documented in the outlier files on the CD.

The variables included in pl.dat are described below.

Sample Identification Variables:

meso	Mesocosm number
group	Treatment group
m	Month
d	Day
y	Year
day	Experiment day

Periphyton Taxonomic Variables:

prep	Periphyton replicate
anki	Ankistrodesmus, cells/mL
chla	Chlamydomonas, cells/mL
sele	Selenastrum, cells/mL
scen	Scenedesmus, cells/mL
schr	Schroederia, cells/mL
ogre	Other green algae, cells/mL

filg	Filamentous green algae, cells/mL
pend	Pennate diatoms, cells/mL
cend	Centric diatoms, cells/mL
melo	Melosira, cells/mL
nost	Nostoc, cells/mL
oscl	Oscillatoria, cells/mL
amoe	Amoeba, cells/mL
eugl	Euglena, cells/mL
afla	Autotrophic flagellates, cells/mL
hfla	Heterotrophic flagellates, cells/mL
gcil	Green ciliate, cells/mL
cil	Ciliates, cells/mL
roti	Rotifers, organisms/mL

Periphyton Functional Variables:

ts	Total solids, mg/mL
tvs	Total volatile solids, mg/mL
afdw	Ash-free dry weight, mg/mL
ttc	TTC respiration, mg/mL
chlo	Chlorophyll, mg/mL

Leaf Pack Taxonomic Variables

lrep	Leaf pack replicate
amph	Amphipods, #/g
cope	Copepods, #/g
ostr	Ostracods, #/g
daph	Daphnia, #/g
snai	Snails, #/g
limp	Limpets, #/g
clam	Clams, #/g
tbug	True bugs, #/g
mayf	Mayflies, #/g
stnf	Stoneflies, #/g
cadf	Caddisflies, #/g
damf	Damselflies, #/g
drag	Dragonflies, #/g
mosq	Mosquitoes, #/g
chir	Chironomids, #/g
bwrm	Bloodworms, #/g
owrm	Other worms, #/g

Summary Statistics and Graphical Analysis

Figures 5 through **45** show each variable plotted by experiment day and group. Most of the periphyton taxa exhibited population increases during the summer. This made pattern analysis difficult because the JP-8 dose was applied in early spring when population numbers were relatively low. The variance associated with the larger summer populations obscured the smaller spring differences related to dose. Because of this, only nonparametric statistical procedures were used to identify patterns (Spearman's rho correlation analysis and Nonparametric Clustering and Association Analysis).

A few taxa exhibited seasonal decreases in population numbers. Diatoms (Centric, Melosira, and Pennate; **Figures 9-11**) had higher populations in early spring, which is consistent with algal bloom sequences for natural populations. Similarly, Ankistrodesmus decreased by mid-summer (Figure 15). Prescott (1962) describes this species as "common pioneers" in artificial ponds and laboratory aquaria, indicating species propensity to bloom early in the absence of

competition from other algal taxa. Schroederia, Selenastrum, and rotifer numbers also decreased during the summer and fall.

Most of the macroinvertebrate taxa were only collected in small numbers on the leaf packs, so the patterns and correlations in these taxa are questionable. Amphipods and snails were relatively abundant and positively correlated. Both increased in abundance as the season progressed; neither exhibited a dose/response relationship. Annelids ("other") were also relatively abundant on the leaf packs, and exhibited a strong, negative correlation with dose (see below).

Correlations
The variables were examined for pair-wise correlations using nonparametric, rank-order Spearman's rho correlation analysis using the SPSS-X NONPAR CORR procedure (SPSS, 1990). These results are shown in Table 1. Because of the large number of samples in the data set, there were many statistically significant correlations ($p >= 0.05$). Correlations that explain at least 60% of the variance between the two variables were plotted in Figures 46 - 60. Most of the positive correlations were simple, indirect measurements of algal population dynamics: during the summer many algal taxa increased in numbers. There were negative correlations between autotrophic vs. heterotrophic flagellates and autotrophic flagellates vs. filamentous green algae, which might have been due to competition. There were also significant, but unremarkable correlations between total community respiration (TTC respiration), chlorophyll, and other indirect measures of biomass (total solids, ash-free dry weight, etc.)

Table 2 lists the taxa that were significantly correlated with dose during the first 60 days of the experiment. Prominently featured on this list were taxa that responded to the JP-8 WSF in the SAM experiments, including Ankistrodesmus, Chlamydomonas, Selenastrum, and Daphnia. Most of the dose/response relationships were negative, indicating inhibition by the jet fuel. The highest correlation coefficient was -0.35 for "other" worms (Annelids). Chlamydomonas, Euglenoids, Rotifers, and Caddisflies, however, responded positively with dose.

Phytoplankton and Water Quality Data
Data Set Description
The phytoplankton and water quality data are located in the compact disk provided with this report. The data are in ASCII format in the file ph.dat (all sampling days) and in subfiles for each sampling date (e.g., ph_004.dat). The CD also contains README files that describe the contents of each data set and SPSS command files for producing descriptive statistics and single-day data sets. The verified Mesa spreadsheet files are located on the same CD.

All data were verified using double-entry, followed by subtraction of the duplicate files. Non-zero results were checked against the laboratory bench sheets and entered correctly. The corrected file was reverified by randomly selecting one row from each experiment day and checking all columns against the bench sheet entries. Any computational errors were corrected and the data were plotted to review outliers. Outliers were reverified against benchsheet entries and decisions concerning rejection or acceptance are documented in the outlier files on the enclosed CD.

The variables included in ph.dat are described below.

Sample Identification Variables:
meso	Mesocosm number
group	Treatment group
m	Month
d	Day

y	Year
day	Experimentday

Water Quality Variables:

pH	pH
amDO1	Initial morning dissolved oxygen, mg/L
pmDO2	Afternoon dissolved oxygen, mg/L
amDO3	Next day morning dissolved oxygen, mg/L
light	AmbientlLight, fc
temp	Water temperature, C
alk	Alkalinity,mg/L
cond	Conductivity, uS
hard	Hardness, mg/L
turb	Turbidity,NTU
no3	Nitrate/nitrite,mg/L
srp	Soluble phosphate, mg/L

Derived Variables:

photo	Photosynthesis,amdo3-amdo1
respir	Respiration,pmdo2-amdo3

Phytoplankton Taxonomic Variables: _

anki	Ankistrodesmus, cells/mL
chla	Chlamydomonas,cells/mL
sele	Selenastrum,cells/mL
scen	Scenedesmus, cells/mL
schro	Schroederia, cells/mL
ougr	Other green algae, cells/mL
filgr	Filamentous green algae, cells/mL
pen	Pennate diatoms,cells/mL
cent	Centric diatoms, cells/mL
nost	Nostoc, cells/mL
osc	Oscillatoria,cells/mL
amoeba	Amoeba,cells/mL
eugl	Euglena,cells/mL
hflag	Heterotrophic flagellates, cells/mL
hcil	Ciliates,cells/mL
rotif	Rotifers, organisms/mL

Phytoplankton Functional Variables:

ts	Total solids, mg/mL
tvs	Total volatile solids, mg/mL
afdw	Ash-free dry weight, mg/mL
ttc	TTCrespiration,mg/mL
chlo	Chlorophyll,mg/mL
toc	Total organic carbon, mg/mL

Summary Statistics and Graphical Analysis

Figures 61 through **96** show each variable plotted by experiment day and group. Fewer taxa showed seasonal increases than in the periphyton data. Many of the taxa either maintained fluctuating populations throughout the experiment or showed a seasonal decrease. Taxa that decreased in the summer included ciliates, centric and pennate diatoms, heterotrophic flagellates, Ankistrodesmus, and Schroederia.

The water quality variables followed predictable patterns associated with seasonal influences. Ambient light and water temperatures were highest during the summer. Respiration increased throughout the year, regardless of whether it was measured as lower morning dissolved oxygen concentrations (amDO1, amDO2), calculated "Respiration," or TTC respiration. Photosynthetic rates (pmDO2), conductivity, and turbidity also increased during the summer. Nitrogen and phosphorus concentrations remained low throughout the experiment, and alkalinity, hardness, and pH remained more-or-less constant.

As with the phytoplankton and leaf pack data, the variance was proportional to the mean for most parameters. Only nonparametric statistical procedures were used to identify patterns (Spearman's rho correlation analysis and Nonparametric Clustering and Association Analysis).

Correlations

The variables were examined for pair-wise correlations using nonparametric, rank-order Spearman's rho correlation analysis using the SPSS-X NONPAR CORR procedure (SPSS, 1990). These results are shown in Table 3. Because of the large number of samples in the data set, there were many statistically significant correlations ($p >= 0.05$). Correlations that explain at least 60% of the variance between the two variables were plotted in Figures 97 through 123.

Most of the positive correlations were simple, indirect measurements of algal population dynamics: during the summer many algal taxa increased in numbers. There were also simple correlations between biologically related measurements. For example, all of the dissolved oxygen measurements were closely correlated with each other and with pH (**Figures 102-103, 106, 115-117**). Similarly, oxygen concentrations were negatively correlated with TTC respiration (**Figures 105, 108, 110**)

Morning dissolved oxygen (**Figure 104** and **107**) was negatively correlated with soluble reactive phosphate. Low morning oxygen concentrations indicate respiration, which consumes nutrients, so this correlation is the reverse of the expected pattern. However, the oxygen concentrations may have been low enough to allow some overnight release of phosphorus. Also, dissolved phosphorus is released during decomposition of organic matter, so the nighttime respiration could be releasing phosphorus from decomposing algae.

Table 4 lists the taxa that were significantly correlated with dose during the first 60 days of the experiment. All of these correlations were relatively weak considering the large sample sizes.

Riffle Results

The periphyton/leaf pack and phytoplankton/water quality data sets were analyzed for multivariate patterns using RIFFLE, a nonmetric clustering and association analysis software program developed by Matthews and Hearne (1991). Riffle cluster qualities and chi-squared association analysis results for each experiment day are shown in **Figures 124-129**. Most of the periphyton/leaf pack data clustered better than randomized data and were significantly associated with treatment group (**Figures 124-145**). In the phytoplankton/water quality data set, very few days clustered better than random, and the associations between RIFFLE clusters and treatment groups were rarely significant. When the water quality data were examined independently (without phytoplankton), there were more dates with good RIFFLE clusters, but still very few significant associations. The cluster strength increased as summer progressed, indicating that groups of mesocosms were diverging from the rest. This was very likely due to microclimatic differences in abiotic factors such as light and temperature, which resulted in "block" effects despite our efforts to reduce such patterns.

Chromatography Data
 Dosing ranges from 0-0.25 mL of neat JP8 per liter of mesocosm water. The highest dose (0.25 mg/L JP8) produced GC curves that were 78% and 110% of the curves produced for low- and high-molecular wt compounds, respectively, in the 15% WSF SAMS. Therefore, the theoretical doses in the mesocosms cover the same ranges as in the SAMS.

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65 Diatoms, Pennate
66 Flagellates, Euglenoids
67 Flagellates, Heterotrophic
68 Green Algae, Ankistrodesmus
69 Green Algae, Chlamydomonas
70 Green Algae, Filamentous
71 Green Algae, Other
72 Green Algae, Scenedesmus
73 Green Algae, Schroederia
74 Green Algae, Selenastrum
75 Miscellaneous Periphyton, Amoebas
76 Miscellaneous Periphyton, Rotifers
77 Phytoplankton Ash-Free Dry Weight
78 Phytoplankton Chlorophyll
79 Phytoplankton Total Organic Carbon
80 Phytoplankton Total Solids
81 Phytoplankton Total Volatile Solids

Table 1. Periphyton and Leaf Pack Correlations, Two-Tailed Spearman's Rho

	ANKI	CHLA	SELE	SCEN	SCHR	OGRE	FILG	PEND	CEND	MELO
CHLA	-.0193									
	N(1917)									
	SIG .398									
SELE	.4217	.0655								
	N(1917)	N(1917)								
	SIG .000	SIG .004								
SCEN	.0425	.1571	.0818							
	N(1917)	N(1917)	N(1917)							
	SIG .063	SIG .000	SIG .000							
SCHR	.8595	-.1143	.4221	.0491						
	N(1917)	N(1917)	N(1917)	N(1917)						
	SIG .000	SIG .000	SIG .000	SIG .031						
OGRE	-.3461	.2050	-.1385	.1059	-.3557					
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)					
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000					
FILG	-.0082	.3603	-.0702	.0371	-.1829	.0686				
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)				
	SIG .720	SIG .000	SIG .002	SIG .104	SIG .000	SIG .003				
PEN	.7827	.0400	.3534	.1278	.6956	-.2721	.1699			
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)			
	SIG .000	SIG .080	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000			
CEND	.3926	-.0227	.2523	.0534	.4020	-.1512	-.0534	.3482		
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)		
	SIG .000	SIG .321	SIG .000	SIG .019	SIG .000	SIG .000	SIG .019	SIG .000		
MELO	.0540	.0512	.0454	.0060	.0341	.0069	.0612	.0277	-.0089	
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	
	SIG .018	SIG .025	SIG .047	SIG .794	SIG .136	SIG .761	SIG .007	SIG .226	SIG .696	
NOST	-.1381	.3322	-.1246	.0459	-.3153	.1114	.6966	.0922	-.1011	.0288
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)
	SIG .000	SIG .000	SIG .000	SIG .044	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .207
OSCL	-.3426	.1646	-.1413	.0925	-.3366	.2103	.4305	-.1263	-.1385	.0538
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .019
AMOE	-.0893	.1169	-.0668	.0423	-.1033	.1427	.1452	.0192	-.0773	.0279
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)
	SIG .000	SIG .000	SIG .003	SIG .064	SIG .000	SIG .000	SIG .000	SIG .401	SIG .001	SIG .223
EUGL	.0077	.1868	.0221	.1382	-.0388	.0553	.1566	.0900	-.0166	.0235
	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)	N(1917)
	SIG .736	SIG .000	SIG .333	SIG .000	SIG .090	SIG .015	SIG .000	SIG .000	SIG .468	SIG .304
AFLA	.1146	.3400	.0023	-.0118	-.0124	-.0541	.6105	.1925	.0122	.0597
	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)
	SIG .000	SIG .000	SIG .925	SIG .622	SIG .606	SIG .024	SIG .000	SIG .000	SIG .612	SIG .013
HFLA	.0272	.3748	-.0426	-.0065	-.1000	.0893	.5601	.0909	-.0252	.0388
	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)
	SIG .257	SIG .000	SIG .076	SIG .788	SIG .000	SIG .000	SIG .000	SIG .000	SIG .293	SIG .106
GCIL	-.0213	.2207	-.0467	.0735	-.0966	.0577	.4742	.1411	-.0356	.0275
	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)	N(1685)
	SIG .381	SIG .000	SIG .056	SIG .003	SIG .000	SIG .018	SIG .000	SIG .000	SIG .144	SIG .260
CIL	-.0145	.1872	.0094	.1560	-.0143	.1342	.4569	.1547	.0087	.0212
	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)
	SIG .547	SIG .000	SIG .697	SIG .000	SIG .550	SIG .000	SIG .000	SIG .000	SIG .717	SIG .376
ROTI	.5834	.0725	.2981	.1225	.5674	-.1844	.1418	.5884	.2854	.0645
	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)	N(1738)
	SIG .000	SIG .002	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .007
TS	.2947	.1119	.1167	.1141	.2179	-.0829	.4771	.4793	.1651	.0403
	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)	N(1915)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .078
TVS	.1782	.2323	.0809	.1166	.0807	.0002	.5695	.3726	.0812	.0498
	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)	N(1910)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .994	SIG .000	SIG .000	SIG .000	SIG .030

	ANKI	CHLA	SELE	SCEN	SCHR	OGRE	FILG	PEND	CEND	MELO
AFDW	.3445	-.0312	.1261	.0776	.2939	-.1220	.2170	.4493	.1962	.0214
	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)	N(1911)
	SIG .000	SIG .173	SIG .000	SIG .001	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .351
TTC	.4893	.2514	.2072	.1444	.4394	-.0839	.5584	.6212	.2236	.0905
	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)	N(1815)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000
CHLO	.1427	.3467	.0193	.1311	.0525	.0896	.6933	.3307	.0407	.0643
	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)	N(1909)
	SIG .000	SIG .000	SIG .399	SIG .000	SIG .022	SIG .000	SIG .000	SIG .000	SIG .076	SIG .005
LREP	.0008	-.0260	.0001	-.0282	-.0008	-.0573	-.0237	-.0299	-.0073	.0067
	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)	N(1916)
	SIG .972	SIG .255	SIG .995	SIG .217	SIG .973	SIG .012	SIG .300	SIG .190	SIG .748	SIG .770
AMPH	-.5984	.0774	-.2968	.0167	-.5806	.3338	.1137	-.4123	-.2271	-.0132
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .003	SIG .000	SIG .527	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .617
COPE	-.1748	.1450	-.1450	-.0692	-.2187	.0900	.2798	-.1146	-.0905	.0395
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .000	SIG .000	SIG .009	SIG .000	SIG .001	SIG .000	SIG .000	SIG .001	SIG .135
OSTR	.1648	.0699	.0667	.0308	.1195	-.0092	.0843	.1274	.0226	.0248
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .008	SIG .011	SIG .244	SIG .000	SIG .727	SIG .001	SIG .000	SIG .392	SIG .348
DAPH	.0230	.1677	-.0646	-.0645	-.1036	-.0678	.4172	.1356	-.0028	.0300
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .383	SIG .000	SIG .014	SIG .015	SIG .000	SIG .010	SIG .000	SIG .000	SIG .916	SIG .256
SNAI	-.6183	-.0198	-.3211	.0198	-.6313	.2103	-.0043	-.4961	-.2490	-.0188
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .455	SIG .000	SIG .453	SIG .000	SIG .000	SIG .871	SIG .000	SIG .000	SIG .476
LIMP	-.0295	-.0767	-.0272	.0593	-.0344	-.0129	.0061	.0051	.0102	.0975
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .264	SIG .004	SIG .303	SIG .025	SIG .192	SIG .624	SIG .817	SIG .848	SIG .699	SIG .000
CLAM	-.2817	-.1230	-.1448	-.1515	-.2343	.1131	-.2174	-.3478	-.1158	-.0342
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .195
TBUG	.0909	.0158	.1018	.0245	.1141	-.0449	-.0627	.0531	-.0193	-.0074
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .001	SIG .549	SIG .000	SIG .353	SIG .000	SIG .089	SIG .018	SIG .044	SIG .464	SIG .779
MAYF	.0266	.0122	-.0083	.0030	.0166	-.0014	.0212	.0154	.0080	-.0093
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .314	SIG .645	SIG .754	SIG .909	SIG .529	SIG .959	SIG .422	SIG .559	SIG .761	SIG .724
STNF	.0215	.0594	-.0193	.0631	.0018	.0285	.0563	.0493	-.0146	-.0056
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .416	SIG .025	SIG .465	SIG .017	SIG .945	SIG .281	SIG .033	SIG .062	SIG .581	SIG .832
CADF	.1513	.0117	.0494	-.0101	.1447	-.0889	.0167	.1201	-.0029	.0577
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .657	SIG .062	SIG .703	SIG .000	SIG .001	SIG .527	SIG .000	SIG .913	SIG .029
DAMF	.0877	.0354	.0128	.0281	.0347	.0229	.0586	.0601	.1462	-.0069
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .001	SIG .180	SIG .628	SIG .287	SIG .189	SIG .386	SIG .026	SIG .023	SIG .000	SIG .794
DRAG	.0364	-.0410	-.0097	.0402	.0623	-.0016	-.0282	.0328	-.0075	-.0028
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .169	SIG .121	SIG .714	SIG .128	SIG .018	SIG .951	SIG .286	SIG .214	SIG .776	SIG .916
MOSQ	-.0535	.0283	-.0468	-.0760	-.0512	.0009	.0553	-.0722	-.0383	.0152
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .043	SIG .284	SIG .076	SIG .004	SIG .052	SIG .972	SIG .036	SIG .006	SIG .147	SIG .565
CHIR	.1672	.0284	.0367	-.0229	.1733	-.0770	.0957	.1665	.0682	-.0009
	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)	N(1434)
	SIG .000	SIG .283	SIG .165	SIG .386	SIG .000	SIG .004	SIG .000	SIG .000	SIG .010	SIG .972
BWRM	.1832	-.0414	.0320	.0100	.2217	-.0858	-.0636	.1219	.0037	.0367
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .000	SIG .117	SIG .226	SIG .706	SIG .000	SIG .001	SIG .016	SIG .000	SIG .888	SIG .165
OWRM	.0243	.1119	.0119	-.0140	-.0339	.0357	.3116	.0960	.0035	.0378
	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)	N(1435)
	SIG .357	SIG .000	SIG .652	SIG .597	SIG .199	SIG .176	SIG .000	SIG .000	SIG .895	SIG .153

	NOST	OSCL	AMOE	EUGL	AFLA	HFLA	GCIL	CIL	ROTI	TS
OSCL	.4248 N(1917) SIG .000									
AMOE	.1652 N(1917) SIG .000	.1758 N(1917) SIG .000								
EUGL	.1472 N(1917) SIG .000	.1519 N(1917) SIG .000	.1240 N(1917) SIG .000							
AFLA	.4944 N(1738) SIG .000	.2841 N(1738) SIG .000	.0775 N(1738) SIG .001	.1325 N(1738) SIG .000						
HFLA	.4992 N(1738) SIG .000	.2451 N(1738) SIG .000	.1109 N(1738) SIG .000	.1251 N(1738) SIG .000	.6861 N(1738) SIG .000					
GCIL	.4269 N(1685) SIG .000	.2954 N(1685) SIG .000	.1257 N(1685) SIG .000	.1470 N(1685) SIG .000	.4532 N(1685) SIG .000	.3281 N(1685) SIG .000				
CIL	.3569 N(1738) SIG .000	.3956 N(1738) SIG .000	:1441 N(1738) SIG .000	.1317 N(1738) SIG .000	.3268 N(1738) SIG .000	.4029 N(1738) SIG .000	.5179 N(1685) SIG .000			
ROTI	.0611 N(1738) SIG .011	-.0396 N(1738) SIG .099	-.0321 N(1738) SIG .181	.1010 N(1738) SIG .000	.1276 N(1738) SIG .000	.0704 N(1738) SIG .003	.0654 N(1685) SIG .007	.2871 N(1738) SIG .000		
TS	.3849 N(1915) SIG .000	.2130 N(1915) SIG .000	.1099 N(1915) SIG .000	.1445 N(1915) SIG .000	.3258 N(1736) SIG .000	.1923 N(1736) SIG .000	.3310 N(1683) SIG .000	.4207 N(1736) SIG .000	.4430 N(1736) SIG .000	
TVS	.4914 N(1910) SIG .000	.2971 N(1910) SIG .000	.1113 N(1910) SIG .000	.1191 N(1910) SIG .000	.3851 N(1732) SIG .000	.2546 N(1732) SIG .000	.3705 N(1680) SIG .000	.4478 N(1732) SIG .000	.3596 N(1732) SIG .000	.7630 N(2004) SIG .000
AFDW	.1559 N(1911) SIG .000	.0218 N(1911) SIG .340	.0836 N(1911) SIG .000	.1165 N(1911) SIG .000	.1327 N(1733) SIG .000	.0609 N(1733) SIG .011	.1464 N(1681) SIG .000	.2256 N(1733) SIG .000	.3811 N(1733) SIG .000	.8173 N(2005) SIG .000
TTC	.3413 N(1815) SIG .000	.2010 N(1815) SIG .000	.1006 N(1815) SIG .000	.1443 N(1815) SIG .000	.4296 N(1732) SIG .000	.3071 N(1732) SIG .000	.3328 N(1683) SIG .000	.3943 N(1732) SIG .000	.4238 N(1732) SIG .000	.5652 N(1908) SIG .000
CHLO	.5503 N(1909) SIG .000	.4217 N(1909) SIG .000	.1242 N(1909) SIG .000	.1316 N(1909) SIG .000	.5077 N(1730) SIG .000	.4538 N(1730) SIG .000	.4043 N(1677) SIG .000	.5533 N(1730) SIG .000	.3872 N(1730) SIG .000	.5902 N(1907) SIG .000
LREP	-.0080 N(1916) SIG .725	-.0256 N(1916) SIG .262	-.0152 N(1916) SIG .507	.0018 N(1916) SIG .936	-.0336 N(1737) SIG .162	.0003 N(1737) SIG .989	-.0563 N(1684) SIG .021	-.0256 N(1737) SIG .286	-.0054 N(1737) SIG .822	-.0475 N(2009) SIG .033
AMPH	.1494 N(1435) SIG .000	.3000 N(1435) SIG .000	.1231 N(1435) SIG .000	-.0084 N(1435) SIG .750	-.0095 N(1435) SIG .719	.0682 N(1435) SIG .010	.1084 N(1382) SIG .000	.2135 N(1435) SIG .000	-.3332 N(1435) SIG .000	-.1532 N(1527) SIG .000
COPE	.2712 N(1435) SIG .000	.1539 N(1435) SIG .000	.0984 N(1435) SIG .000	.0133 N(1435) SIG .614	.2571 N(1435) SIG .000	.3317 N(1435) SIG .000	.0835 N(1382) SIG .002	.1840 N(1435) SIG .000	-.0274 N(1435) SIG .300	-.0116 N(1527) SIG .652
OSTR	.1155 N(1435) SIG .000	-.0464 N(1435) SIG .079	.0197 N(1435) SIG .455	-.0156 N(1435) SIG .556	.0668 N(1435) SIG .011	.0634 N(1435) SIG .016	.0606 N(1382) SIG .024	-.0196 N(1435) SIG .458	.0775 N(1435) SIG .003	.0618 N(1527) SIG .016
DAPH	.4415 N(1435) SIG .000	.2018 N(1435) SIG .000	.0702 N(1435) SIG .008	.0427 N(1435) SIG .106	.3854 N(1435) SIG .000	.3249 N(1435) SIG .000	.2827 N(1382) SIG .000	.1812 N(1435) SIG .000	-.0069 N(1435) SIG .795	.0888 N(1527) SIG .001
SNAI	.0422 N(1435) SIG .110	.1478 N(1435) SIG .000	.0039 N(1435) SIG .881	-.0117 N(1435) SIG .659	-.1288 N(1435) SIG .000	-.0979 N(1435) SIG .000	.0113 N(1382) SIG .674	.0401 N(1435) SIG .129	-.3784 N(1435) SIG .000	-.2126 N(1527) SIG .000
LIMP	-.0031 N(1435) SIG .907	.0005 N(1435) SIG .986	-.0063 N(1435) SIG .813	.0048 N(1435) SIG .855	-.0394 N(1435) SIG .136	-.0588 N(1435) SIG .026	.0236 N(1382) SIG .380	.0064 N(1435) SIG .809	-.0141 N(1435) SIG .593	.0218 N(1527) SIG .395

	NOST	OSCL	AMOE	EUGL	AFLA	HFLA	GCIL	CIL	ROTI	TS
CLAM	-.2101 N(1435) SIG .000	-.0845 N(1435) SIG .001	-.0214 N(1435) SIG .418	-.0966 N(1435) SIG .000	-.2372 N(1435) SIG .000	-.1567 N(1435) SIG .000	-.1665 N(1382) SIG .000	-.1803 N(1435) SIG .000	-.3056 N(1435) SIG .000	-.2985 N(1527) SIG .000
TBUG	-.0674 N(1435) SIG .011	-.0308 N(1435) SIG .244	-.0333 N(1435) SIG .207	-.0053 N(1435) SIG .842	-.0188 N(1435) SIG .476	-.0525 N(1435) SIG .047	-.0245 N(1382) SIG .363	-.0312 N(1435) SIG .237	.0768 N(1435) SIG .004	.0105 N(1527) SIG .682
MAYF	.0370 N(1435) SIG .161	-.0271 N(1435) SIG .305	-.0153 N(1435) SIG .563	-.0049 N(1435) SIG .853	.0586 N(1435) SIG .026	.0470 N(1435) SIG .075	.0227 N(1382) SIG .400	.0452 N(1435) SIG .087	.0658 N(1435) SIG .013	.0741 N(1527) SIG .004
STNF	.0521 N(1435) SIG .048	-.0092 N(1435) SIG .729	.0236 N(1435) SIG .371	.0415 N(1435) SIG .116	.0247 N(1435) SIG .350	.0613 N(1435) SIG .020	.0080 N(1382) SIG .765	.0133 N(1435) SIG .615	.0030 N(1435) SIG .909	.0296 N(1527) SIG .248
CADF	.0186 N(1435) SIG .481	-.0384 N(1435) SIG .145	.0000 N(1435) SIG .999	.0221 N(1435) SIG .403	.0418 N(1435) SIG .114	.0157 N(1435) SIG .553	.0115 N(1382) SIG .670	.0070 N(1435) SIG .792	.1895 N(1435) SIG .000	.1535 N(1527) SIG .000
DAMF	.0742 N(1435) SIG .005	-.0066 N(1435) SIG .804	-.0156 N(1435) SIG .555	.0127 N(1435) SIG .630	.0663 N(1435) SIG .012	.0516 N(1435) SIG .051	.0473 N(1382) SIG .079	-.0308 N(1435) SIG .243	.0581 N(1435) SIG .028	.0837 N(1527) SIG .001
DRAG	-.0254 N(1435) SIG .336	.0173 N(1435) SIG .513	-.0235 N(1435) SIG .374	.0276 N(1435) SIG .296	-.0028 N(1435) SIG .914	-.0173 N(1435) SIG .514	-.0361 N(1382) SIG .179	-.0125 N(1435) SIG .637	.0419 N(1435) SIG .113	.0180 N(1527) SIG .481
MOSQ	.0645 N(1435) SIG .015	.0473 N(1435) SIG .073	.0273 N(1435) SIG .302	-.0151 N(1435) SIG .568	.0550 N(1435) SIG .037	.0583 N(1435) SIG .027	-.0237 N(1382) SIG .380	.0044 N(1435) SIG .868	-.0548 N(1435) SIG .038	-.0381 N(1527) SIG .137
CHIR	.0750 N(1434) SIG .004	.0684 N(1434) SIG .010	.0582 N(1434) SIG .028	.0265 N(1434) SIG .316	.1320 N(1434) SIG .000	.0684 N(1434) SIG .010	.0301 N(1381) SIG .264	.0398 N(1434) SIG .132	.2020 N(1434) SIG .000	.0636 N(1526) SIG .013
BWRM	-.0873 N(1435) SIG .001	-.0757 N(1435) SIG .004	-.0392 N(1435) SIG .138	-.0260 N(1435) SIG .326	-.0433 N(1435) SIG .101	-.0462 N(1435) SIG .080	-.0524 N(1382) SIG .052	-.0065 N(1435) SIG .806	.1632 N(1435) SIG .000	.1414 N(1527) SIG .000
OWRM	.2472 N(1435) SIG .000	.1522 N(1435) SIG .000	.1305 N(1435) SIG .000	.0840 N(1435) SIG .001	.2545 N(1435) SIG .000	.2819 N(1435) SIG .000	.1636 N(1382) SIG .000	.1597 N(1435) SIG .000	-.0374 N(1435) SIG .157	.0107 N(1527) SIG .676

	TVS	AFDW	TTC	CHLO	LREP	AMPH	COPE	OSTR	DAPH	SNAI
AFDW	.3322 N(2004) SIG .000									
TTC	.6212 N(1903) SIG .000	.3523 N(1904) SIG .000								
CHLO	.6576 N(1902) SIG .000	.3097 N(1903) SIG .000	.6471 N(1807) SIG .000							
LREP	-.0426 N(2003) SIG .057	-.0394 N(2004) SIG .078	-.0423 N(1910) SIG .065	-.0201 N(2005) SIG .368						
AMPH	.0162 N(1522) SIG .526	-.2509 N(1523) SIG .000	-.0818 N(1523) SIG .001	.0201 N(1523) SIG .433	.0301 N(1624) SIG .226					
COPE	.0881 N(1522) SIG .001	-.0863 N(1523) SIG .001	.0811 N(1523) SIG .002	.1507 N(1523) SIG .000	.0031 N(1624) SIG .900	.2630 N(1624) SIG .000				
OSTR	.0651 N(1522) SIG .011	.0612 N(1523) SIG .017	.0957 N(1523) SIG .000	.0367 N(1523) SIG .152	.0349 N(1624) SIG .160	-.0463 N(1624) SIG .062	.0666 N(1624) SIG .007			
DAPH	.1919 N(1522) SIG .000	-.0526 N(1523) SIG .040	.2298 N(1523) SIG .000	.2141 N(1523) SIG .000	-.0095 N(1624) SIG .702	.2062 N(1624) SIG .000	.2583 N(1624) SIG .000	.1452 N(1624) SIG .000		
SNAI	-.0769 N(1522) SIG .003	-.2521 N(1523) SIG .000	-.2471 N(1523) SIG .000	-.1796 N(1523) SIG .000	-.0372 N(1624) SIG .134	.7012 N(1624) SIG .000	.1696 N(1624) SIG .000	-.0286 N(1624) SIG .249	.1359 N(1624) SIG .000	

	TVS	AFDW	TTC	CHLO	LREP	AMPH	COPE	OSTR	DAPH	SNAI
LIMP	-.0028	.0216	.0053	-.0277	-.0346	.0158	.0508	-.0183	.0299	.0782
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .914	SIG .399	SIG .837	SIG .280	SIG .164	SIG .524	**SIG .040**	SIG .461	SIG .228	**SIG .002**
CLAM	-.2473	-.2259	-.2791	-.2940	.0160	.3031	.0619	-.0460	-.0762	.3592
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .000	**SIG .000**	**SIG .000**	**SIG .000**	SIG .519	**SIG .000**	**SIG .013**	SIG .064	**SIG .002**	**SIG .000**
TBUG	.0052	.0126	.0476	-.0148	.0297	-.0828	-.0527	-.0101	-.0502	-.0597
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .840	SIG .622	SIG .063	SIG .565	SIG .232	**SIG .001**	**SIG .034**	SIG .684	**SIG .043**	**SIG .016**
MAYF	.0627	.0613	-.0056	.0363	.0242	-.0323	.0285	-.0142	-.0473	-.0206
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .015	**SIG .017**	SIG .826	SIG .157	SIG .330	SIG .193	SIG .251	SIG .567	SIG .057	SIG .408
STNF	.0516	-.0046	.0265	.0414	.0548	.0151	-.0060	.0656	.0576	.0152
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .044	SIG .859	SIG .302	SIG .106	**SIG .027**	SIG .544	SIG .808	**SIG .008**	**SIG .020**	SIG .542
CADF	.0749	.1449	-.0295	.1121	.0311	-.2114	-.1004	.0110	-.1262	-.2163
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .003	**SIG .000**	SIG .250	**SIG .000**	SIG .211	**SIG .000**	**SIG .000**	SIG .658	**SIG .000**	**SIG .000**
DAMF	.0167	.0989	-.0132	.0572	-.0026	-.0551	-.0392	.0281	-.0028	-.0850
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .514	**SIG .000**	SIG .606	**SIG .026**	SIG .915	**SIG .026**	SIG .114	SIG .257	SIG .909	**SIG .001**
DRAG	-.0354	.0439	-.0525	.0213	.0381	-.0704	-.0527	-.0101	-.0502	-.0778
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .168	SIG .087	**SIG .041**	SIG .406	SIG .125	**SIG .005**	**SIG .034**	SIG .684	**SIG .043**	**SIG .002**
MOSQ	-.0276	-.0644	-.0383	.0223	-.0041	-.0384	.0969	-.0304	.1346	-.0542
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .281	**SIG .012**	SIG .135	SIG .384	SIG .868	SIG .122	**SIG .000**	SIG .221	**SIG .000**	**SIG .029**
CHIR	.0728	.0439	.2032	.1334	.0325	-.1056	-.0145	.0059	.0995	-.1435
	N(1521)	N(1522)	N(1522)	N(1522)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)
	SIG .005	SIG .087	**SIG .000**	**SIG .000**	SIG .190	**SIG .000**	SIG .561	SIG .813	**SIG .000**	**SIG .000**
BWRM	-.0170	.1835	-.0679	.0821	.0415	-.2111	-.0905	.0239	-.1382	-.2470
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .508	**SIG .000**	**SIG .008**	**SIG .001**	SIG .095	**SIG .000**	**SIG .000**	SIG .335	**SIG .000**	**SIG .000**
OWRM	.1009	-.0664	.2289	.1199	.0151	.2580	.1708	.0961	.3583	.2493
	N(1522)	N(1523)	N(1523)	N(1523)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)
	SIG .000	**SIG .010**	**SIG .000**	**SIG .000**	SIG .542	**SIG .000**	**SIG .000**	**SIG .000**	**SIG .000**	**SIG .000**

	LIMP	CLAM	TBUG	MAYF	STNF	CADF	DAMF	DRAG	MOSQ	CHIR
CLAM	.0595									
	N(1624)									
	SIG .017									
TBUG	-.0079	-.0287								
	N(1624)	N(1624)								
	SIG .751	SIG .248								
MAYF	.0454	-.0406	-.0061							
	N(1624)	N(1624)	N(1624)							
	SIG .067	SIG .102	SIG .805							
STNF	-.0067	-.0242	-.0037	-.0052						
	N(1624)	N(1624)	N(1624)	N(1624)						
	SIG .788	SIG .330	SIG .883	SIG .835						
CADF	-.0099	-.1065	.0212	.0318	-.0144					
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)					
	SIG .689	**SIG .000**	SIG .394	SIG .200	SIG .563					
DAMF	-.0116	-.0260	-.0064	-.0090	-.0054	.1370				
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)				
	SIG .641	SIG .295	SIG .798	SIG .717	SIG .829	**SIG .000**				
DRAG	-.0079	-.0287	-.0043	-.0061	-.0037	.1437	-.0064			
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)			
	SIG .751	SIG .248	SIG .862	SIG .806	SIG .883	**SIG .000**	SIG .798			

	LIMP	CLAM	TBUG	MAYF	STNF	CADF	DAMF	DRAG	MOSQ	CHIR
MOSQ	.0042	-.0616	.0354	-.0186	-.0111	.0143	-.0192	-.0131		
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)		
	SIG .866	**SIG .013**	SIG .154	SIG .454	SIG .656	SIG .564	SIG .439	SIG .598		
CHIR	-.0138	-.1224	.0881	-.0149	-.0204	.0350	.0614	.0344	.0463	
	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	N(1623)	
	SIG .577	**SIG .000**	**SIG .000**	SIG .548	SIG .411	SIG .159	**SIG .013**	SIG .166	SIG .062	
BWRM	.0202	-.0985	-.0149	.0399	-.0125	.2004	.0675	.0727	.0431	-.0090
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1623)
	SIG .415	**SIG .000**	SIG .549	SIG .108	SIG .614	**SIG .000**	**SIG .007**	**SIG .003**	SIG .082	SIG .717
OWRM	.0709	.0466	-.0458	.0334	.0052	-.1476	-.0481	-.0547	.0275	.1314
	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1624)	N(1623)
	SIG .004	SIG .060	SIG .065	SIG .178	SIG .834	**SIG .000**	SIG .052	**SIG .027**	SIG .269	**SIG .000**

	BWRM
OWRM	-.1312
	N(1624)
	SIG .000

Abbreviations:

anki	Ankistrodesmus
chla	Chlamydomonas
sele	Selenastrum
scen	Scenedesmus
schr	Schroederia
ogre	Other Green Algae
filg	Filamentous Green Algae
pend	Pennate Diatoms
cend	Centric Diatoms
melo	Melosira
nost	Nostoc
oscl	Oscillatoria
amoe	Amoebas
eugl	Euglenoids
afla	Autotrophic Flagellates
hfla	Heterotrophic Flagellates
gcil	Ciliates, Algal Grazer (Green)
cil	Other Ciliates
roti	Rotifers
ts	Total Solids
tvs	Total Volatile Solids
afdw	Ash-Free Dry Weight
ttc	TTC Respiration
chlo	Chlorophyll
lrep	Leaf Pack Replicate
amph	Amphipods
cope	Copepods
ostr	Ostracods
daph	Daphnia
snai	Snails
limp	Limpets
clam	Clams
tbug	Hemiptera (True bugs)
mayf	Ephemeroptera (Mayflies)
stnf	Plecoptera (Stoneflies)
cadf	Trichoptera (Caddisflies)
damf	Odonata, Damselflies
drag	Odonata, Dragonflies
mosq	Diptera, Mosquitoes
chir	Diptera, Chironomids
bwrm	Bloodworms
owrm	Other Worms

Table 2. Periphyton and Leaf Pack Variables Significantly Correlated With Dose.

	Spearman's rho	N	SIG
Ankistrodesmus	-.1779	765	0.000
Chlamydomonas	.0883	765	0.015
Selenastrum	-.1097	765	0.002
Schroederia	-.1330	765	0.000
Filamentous Greens	-.1015	765	0.005
Pennate Diatoms	-.2124	765	0.000
Flagellates, Autotr.	-.1163	682	0.002
Flagellates, Euglenoids	.0890	765	0.014
Ciliates (Green)	-.1258	629	0.002
Rotifers	.0955	682	0.013
Total Solids	-.0728	764	0.044
Total Vol. Solids	-.1381	762	0.000
TTC Respiration	-.1007	761	0.005
Daphnia	-.1872	383	0.000
Caddisflies	.1157	383	0.024
Other Worms	-.3465	383	0.000

Table 3. Phytoplankton and Water Quality Correlations, Tow-Tailed Spearman's Rho.

	PH	AMDO1	PMDO2	AMDO3	LIGHT	TEMP	ALK	COND	HARD	TURB
AMDO1	.6875 N(672) SIG .000									
PMDO2	.6455 N(671) SIG .000	.8392 N(623) SIG .000								
AMDO3	.7482 N(791) SIG .000	.9396 N(671) SIG .000	.8361 N(670) SIG .000							
LIGHT	.0248 N(744) SIG .500	.2679 N(648) SIG .000	.2832 N(623) SIG .000	.2078 N(743) SIG .000						
TEMP	-.2150 N(768) SIG .000	-.4131 N(672) SIG .000	-.1542 N(647) SIG .000	-.4801 N(767) SIG .000	.0424 N(744) SIG .248					
ALK	-.2167 N(528) SIG .000	-.2127 N(432) SIG .000	-.2333 N(432) SIG .000	-.3067 N(527) SIG .000	-.1232 N(480) SIG .007	.1937 N(504) SIG .000				
COND	-.2942 N(792) SIG .000	-.3348 N(672) SIG .000	-.3243 N(671) SIG .000	-.3606 N(791) SIG .000	-.3153 N(744) SIG .000	-.0982 N(768) SIG .006	.3838 N(528) SIG .000			
HARD	-.2406 N(528) SIG .000	-.2689 N(432) SIG .000	-.3179 N(432) SIG .000	-.2948 N(527) SIG .000	-.2553 N(480) SIG .000	-.1053 N(504) SIG .018	.2231 N(528) SIG .000	.5445 N(528) SIG .000		
TURB	-.3803 N(527) SIG .000	-.4151 N(431) SIG .000	-.4206 N(431) SIG .000	-.3964 N(526) SIG .000	-.3468 N(479) SIG .000	-.0270 N(503) SIG .546	.2042 N(527) SIG .000	.5483 N(527) SIG .000	.3717 N(527) SIG .000	
NO3	.1383 N(527) SIG .001	.3102 N(431) SIG .000	.1596 N(431) SIG .001	.2344 N(526) SIG .000	.0734 N(479) SIG .108	.1577 N(503) SIG .000	.0917 N(527) SIG .035	.1512 N(527) SIG .000	-.0775 N(527) SIG .076	-.0221 N(526) SIG .614
SRP	-.4785 N(528) SIG .000	-.6759 N(432) SIG .000	-.6408 N(432) SIG .000	-.6414 N(527) SIG .000	-.1579 N(480) SIG .001	.2230 N(504) SIG .000	.1730 N(528) SIG .000	.3663 N(528) SIG .000	.2050 N(528) SIG .000	.2911 N(527) SIG .000
PHOTO	.1025 N(671) SIG .008	-.0313 N(671) SIG .419	.0562 N(622) SIG .161	.2338 N(671) SIG .000	-.0587 N(647) SIG .136	-.3107 N(671) SIG .000	.0365 N(431) SIG .450	.1579 N(671) SIG .000	.0478 N(431) SIG .322	-.0318 N(430) SIG .510
RESPIR	.0817 N(670) SIG .035	.2025 N(622) SIG .000	.5309 N(670) SIG .000	.0188 N(670) SIG .628	.1979 N(622) SIG .000	.4111 N(646) SIG .000	.1342 N(431) SIG .005	-.1046 N(670) SIG .007	-.0715 N(431) SIG .138	-.2689 N(430) SIG .000
ANKI	.5580 N(478) SIG .000	.5822 N(407) SIG .000	.4421 N(407) SIG .000	.6157 N(477) SIG .000	-.0576 N(454) SIG .221	-.2066 N(478) SIG .000	-.1820 N(478) SIG .000	-.5698 N(478) SIG .000	-.2276 N(478) SIG .000	-.3684 N(477) SIG .000
CHLA	.3644 N(477) SIG .000	.3207 N(406) SIG .000	.3439 N(406) SIG .000	.3329 N(476) SIG .000	-.0316 N(453) SIG .502	.1396 N(477) SIG .002	-.0937 N(477) SIG .041	-.2947 N(477) SIG .000	-.2564 N(477) SIG .000	-.2653 N(476) SIG .000
SELE	.2590 N(478) SIG .000	.2982 N(407) SIG .000	.2449 N(407) SIG .000	.2884 N(477) SIG .000	.0115 N(454) SIG .807	-.0615 N(478) SIG .180	-.1217 N(478) SIG .008	-.1860 N(478) SIG .000	-.0575 N(478) SIG .209	-.0899 N(477) SIG .050
SCEN	.0052 N(479) SIG .910	.0045 N(407) SIG .928	.0482 N(407) SIG .332	-.0525 N(478) SIG .252	-.1318 N(455) SIG .005	.0030 N(479) SIG .948	.0171 N(479) SIG .709	.1315 N(479) SIG .004	.1313 N(479) SIG .004	.1405 N(478) SIG .002
SCHRO	.3839 N(479) SIG .000	.4020 N(407) SIG .000	.2423 N(407) SIG .000	.4133 N(478) SIG .000	-.1454 N(455) SIG .002	-.1821 N(479) SIG .000	-.1540 N(479) SIG .001	-.5900 N(479) SIG .000	-.1515 N(479) SIG .001	-.2408 N(478) SIG .000
OUGR	.0262 N(479) SIG .567	-.0452 N(407) SIG .363	-.0066 N(407) SIG .895	-.0400 N(478) SIG .383	-.0179 N(455) SIG .703	.0982 N(479) SIG .032	.1700 N(479) SIG .000	.1386 N(479) SIG .002	.0448 N(479) SIG .328	.0352 N(478) SIG .443
FILGR	.3809 N(479) SIG .000	.3238 N(407) SIG .000	.3938 N(407) SIG .000	.4175 N(478) SIG .000	.2041 N(455) SIG .000	.0344 N(479) SIG .453	-.1127 N(479) SIG .014	-.0766 N(479) SIG .094	-.1921 N(479) SIG .000	-.2537 N(478) SIG .000
PEN	.6209 N(479) SIG .000	.5701 N(407) SIG .000	.5404 N(407) SIG .000	.6411 N(478) SIG .000	.0467 N(455) SIG .321	-.0895 N(479) SIG .050	-.1735 N(479) SIG .000	-.5220 N(479) SIG .000	-.2534 N(479) SIG .000	-.4295 N(478) SIG .000

	PH	AMDO1	PMDO2	AMDO3	LIGHT	TEMP	ALK	COND	HARD	TURB
CENT	.1772	.2008	.1780	.1513	-.0551	-.0854	-.1516	-.1585	-.0692	-.1219
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .000	SIG .000	SIG .000	SIG .001	SIG .241	SIG .062	SIG .001	SIG .001	SIG .130	SIG .008
NOST	.1404	.0506	.2433	.1143	.2216	.2014	-.0131	-.1025	-.1818	-.1881
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .002	SIG .309	SIG .000	SIG .012	SIG .000	SIG .000	SIG .775	SIG .025	SIG .000	SIG .000
OSC	-.0262	-.0927	.0031	-.0835	-.0642	.0986	-.0891	-.1102	-.0287	-.0829
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .568	SIG .062	SIG .950	SIG .068	SIG .171	SIG .031	SIG .051	SIG .016	SIG .530	SIG .070
AMOEBA	-.1883	-.2306	-.1337	-.2285	.1540	.2100	.0222	-.1029	-.0971	-.1626
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .000	SIG .000	SIG .007	SIG .000	SIG .001	SIG .000	SIG .628	SIG .024	SIG .034	SIG .000
EUGL	.0603	.0246	.1590	.0707	.2981	.2147	.0655	-.0076	-.1733	-.2272
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .188	SIG .620	SIG .001	SIG .123	SIG .000	SIG .000	SIG .152	SIG .868	SIG .000	SIG .000
HFLAG	.3136	.2974	.2694	.3153	-.0623	-.1937	-.2457	-.6039	-.2584	-.3619
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .185	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000
HCIL	.3133	.3748	.3908	.3839	-.0054	-.2334	-.2278	-.5204	-.1827	-.2168
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .908	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000
ROTIF	.0621	.1001	.1283	.0911	-.0504	-.0831	-.2321	-.2570	-.0275	-.0528
	N(479)	N(407)	N(407)	N(478)	N(455)	N(479)	N(479)	N(479)	N(479)	N(478)
	SIG .175	SIG .044	SIG .010	SIG .047	SIG .283	SIG .069	SIG .000	SIG .000	SIG .548	SIG .249
TS	-.1766	-.1917	-.1269	-.3388	-.0242	.4068	.2041	.2517	.1031	-.0967
	N(504)	N(408)	N(432)	N(503)	N(456)	N(480)	N(504)	N(504)	N(504)	N(503)
	SIG .000	SIG .000	SIG .008	SIG .000	SIG .606	SIG .000	SIG .000	SIG .000	SIG .021	SIG .030
TVS	-.3201	-.1320	-.1397	-.3107	-.0562	.0629	.1888	.3689	.2985	.1543
	N(503)	N(407)	N(431)	N(502)	N(455)	N(479)	N(503)	N(503)	N(503)	N(502)
	SIG .000	SIG .008	SIG .004	SIG .000	SIG .232	SIG .169	SIG .000	SIG .000	SIG .000	SIG .001
AFDW	-.0455	-.0777	-.0192	-.2052	.0564	.4215	.1833	.1804	-.0198	-.2118
	N(503)	N(407)	N(431)	N(502)	N(455)	N(479)	N(503)	N(503)	N(503)	N(502)
	SIG .308	SIG .118	SIG .692	SIG .000	SIG .230	SIG .000	SIG .000	SIG .000	SIG .657	SIG .000
TTC	-.6993	-.6356	-.7786	-.7048	-.1532	-.1234	.2479	.5983	.3814	.5525
	N(479)	N(383)	N(407)	N(478)	N(431)	N(455)	N(479)	N(479)	N(479)	N(478)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .001	SIG .008	SIG .000	SIG .000	SIG .000	SIG .000
CHLO	.3417	.3874	.4671	.3231	.1455	.0925	-.1526	-.3345	-.3107	-.2402
	N(523)	N(427)	N(427)	N(522)	N(478)	N(499)	N(523)	N(523)	N(523)	N(522)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .001	SIG .039	SIG .000	SIG .000	SIG .000	SIG .000
TOC	-.2876	-.1828	-.4230	-.2381	-.2233	-.1529	.0143	-.1512	.0901	.2711
	N(434)	N(370)	N(366)	N(433)	N(410)	N(434)	N(434)	N(434)	N(434)	N(433)
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000	SIG .001	SIG .766	SIG .002	SIG .061	SIG .000

	NO3	SRP	PHOTO	RESPIR	ANKI	CHLA	SELE	SCEN	SCHRO	OUGR
SRP	-.2913									
	N(527)									
	SIG .000									
PHOTO	-.2593	.1420								
	N(430)	N(431)								
	SIG .000	SIG .003								
RESPIR	.2328	-.2268	-.3514							
	N(430)	N(431)	N(622)							
	SIG .000	SIG .000	SIG .000							
ANKI	.0766	-.4085	.1364	-.0867						
	N(477)	N(478)	N(406)	N(406)						
	SIG .095	SIG .000	SIG .006	SIG .081						
CHLA	.1786	-.2953	-.1844	.2006	.4194					
	N(476)	N(477)	N(405)	N(405)	N(477)					
	SIG .000	SIG .000	SIG .000	SIG .000	SIG .000					
SELE	.0304	-.2271	-.0489	.0055	.3382	.2465				
	N(477)	N(478)	N(406)	N(406)	N(478)	N(477)				
	SIG .508	SIG .000	SIG .325	SIG .912	SIG .000	SIG .000				

	NO3	SRP	PHOTO	RESPIR	ANKI	CHLA	SELE	SCEN	SCHRO	OUGR
SCEN	-.0374 N(478) SIG .414	-.0145 N(479) SIG .752	-.0201 N(406) SIG .687	.1145 N(406) SIG .021	-.0928 N(478) SIG .043	.0161 N(477) SIG .725	.1541 N(478) SIG .001			
SCHRO	-.1451 N(478) SIG .001	-.2909 N(479) SIG .000	.1679 N(406) SIG .001	-.1870 N(406) SIG .000	.8406 N(478) SIG .000	.2850 N(477) SIG .000	.2504 N(478) SIG .000	-.0234 N(479) SIG .609		
OUGR	.1876 N(478) SIG .000	-.0017 N(479) SIG .970	-.0871 N(406) SIG .080	.1113 N(406) SIG .025	-.0258 N(478) SIG .574	.0541 N(477) SIG .238	.0199 N(478) SIG .665	.1817 N(479) SIG .000	-.0793 N(479) SIG .083	
FILGR	.2976 N(478) SIG .000	-.2962 N(479) SIG .000	-.0535 N(406) SIG .282	.2048 N(406) SIG .000	.2588 N(478) SIG .000	.2919 N(477) SIG .000	.2226 N(478) SIG .000	-.1055 N(479) SIG .021	.0420 N(479) SIG .360	.0315 N(479) SIG .491
PEN	.1529 N(478) SIG .001	-.4532 N(479) SIG .000	.0322 N(406) SIG .517	.1609 N(406) SIG .001	.7974 N(478) SIG .000	.4750 N(477) SIG .000	.3424 N(478) SIG .000	-.0762 N(479) SIG .096	.6332 N(479) SIG .000	.0108 N(479) SIG .813
CENT	-.0888 N(478) SIG .052	-.1357 N(479) SIG .003	-.0802 N(406) SIG .107	.0231 N(406) SIG .642	.1744 N(478) SIG .000	.1734 N(477) SIG .000	.1987 N(478) SIG .000	.0533 N(479) SIG .244	.1637 N(479) SIG .000	.0381 N(479) SIG .405
NOST	.1352 N(478) SIG .003	-.1034 N(479) SIG .024	-.1138 N(406) SIG .022	.3710 N(406) SIG .000	.0001 N(478) SIG .998	.1871 N(477) SIG .000	.0690 N(478) SIG .132	.0002 N(479) SIG .996	-.1041 N(479) SIG .023	.0678 N(479) SIG .138
OSC	-.0214 N(478) SIG .641	.0466 N(479) SIG .308	-.1427 N(406) SIG .004	.1174 N(406) SIG .018	-.0235 N(478) SIG .609	-.0109 N(477) SIG .812	.0145 N(478) SIG .751	-.0255 N(479) SIG .578	.0162 N(479) SIG .724	-.0959 N(479) SIG .036
AMOEBA	-.1640 N(478) SIG .000	.1226 N(479) SIG .007	-.0197 N(406) SIG .693	.1560 N(406) SIG .002	-.2486 N(478) SIG .000	-.0399 N(477) SIG .385	-.1170 N(478) SIG .010	.0639 N(479) SIG .163	-.1997 N(479) SIG .000	.1272 N(479) SIG .005
EUGL	.1967 N(478) SIG .000	-.0289 N(479) SIG .528	-.0155 N(406) SIG .756	.3310 N(406) SIG .000	-.1001 N(478) SIG .029	.1618 N(477) SIG .000	.0309 N(478) SIG .501	.0380 N(479) SIG .406	-.2290 N(479) SIG .000	.1777 N(479) SIG .000
HFLAG	-.2943 N(478) SIG .000	-.2383 N(479) SIG .000	.1359 N(406) SIG .006	-.0565 N(406) SIG .256	.6683 N(478) SIG .000	.3713 N(477) SIG .000	.1651 N(478) SIG .000	-.0223 N(479) SIG .626	.7134 N(479) SIG .000	-.1155 N(479) SIG .011
HCIL	-.2988 N(478) SIG .000	-.2525 N(479) SIG .000	.0374 N(406) SIG .453	.0730 N(406) SIG .142	.5230 N(478) SIG .000	.3262 N(477) SIG .000	.2058 N(478) SIG .000	.0295 N(479) SIG .520	.5845 N(479) SIG .000	-.1195 N(479) SIG .009
ROTIF	-.1715 N(478) SIG .000	-.0695 N(479) SIG .129	-.0350 N(406) SIG .482	-.0208 N(406) SIG .676	.2697 N(478) SIG .000	.1699 N(477) SIG .000	.1856 N(478) SIG .000	.0718 N(479) SIG .116	.3397 N(479) SIG .000	-.0647 N(479) SIG .157
TS	.0808 N(503) SIG .070	.0277 N(504) SIG .535	-.1580 N(407) SIG .001	.3584 N(431) SIG .000	-.3224 N(478) SIG .000	.0774 N(477) SIG .091	-.1315 N(478) SIG .004	.1367 N(479) SIG .003	-.2433 N(479) SIG .000	.2200 N(479) SIG .000
TVS	-.0583 N(502) SIG .192	.0050 N(503) SIG .911	-.1148 N(406) SIG .021	.2492 N(430) SIG .000	-.3969 N(477) SIG .000	-.1744 N(476) SIG .000	-.1549 N(477) SIG .001	.1390 N(478) SIG .002	-.2589 N(478) SIG .000	-.0038 N(478) SIG .934
AFDW	.2131 N(502) SIG .000	-.0167 N(503) SIG .708	-.1150 N(406) SIG .020	.3503 N(430) SIG .000	-.1937 N(477) SIG .000	.1749 N(476) SIG .000	-.0614 N(477) SIG .181	.0856 N(478) SIG .061	-.2091 N(478) SIG .000	.2558 N(478) SIG .000
TTC	-.1921 N(478) SIG .000	.6311 N(479) SIG .000	.0600 N(382) SIG .242	-.4248 N(406) SIG .000	-.6367 N(454) SIG .000	-.4597 N(454) SIG .000	-.2439 N(454) SIG .000	.0296 N(455) SIG .528	-.4710 N(455) SIG .000	.0135 N(455) SIG .774
CHLO	.1865 N(522) SIG .000	-.3217 N(523) SIG .000	-.2741 N(426) SIG .000	.3929 N(426) SIG .000	.1703 N(473) SIG .000	.3006 N(472) SIG .000	.1161 N(473) SIG .011	.0240 N(474) SIG .603	.0999 N(474) SIG .030	.1301 N(474) SIG .005
TOC	-.3534 N(433) SIG .000	.1935 N(434) SIG .000	.2435 N(369) SIG .000	-.3638 N(365) SIG .000	.0521 N(433) SIG .279	-.2689 N(433) SIG .000	-.0144 N(433) SIG .765	.1536 N(434) SIG .001	.2664 N(434) SIG .000	-.0797 N(434) SIG .097

	FILGR	PEN	CENT	NOST	OSC	AMOEBA	EUGL	HFLAG	HCIL	ROTIF
PEN	.4012 N(479) **SIG .000**									
CENT	.0334 N(479) SIG .466	.2121 N(479) **SIG .000**								
NOST	.3556 N(479) **SIG .000**	.1359 N(479) SIG .003	-.0154 N(479) SIG .737							
OSC	.0427 N(479) SIG .351	-.0186 N(479) SIG .685	-.0352 N(479) SIG .443	.0869 N(479) SIG .057						
AMOEBA	-.0887 N(479) SIG .052	-.1527 N(479) **SIG .001**	-.0558 N(479) SIG .222	.1789 N(479) **SIG .000**	.1162 N(479) **SIG .011**					
EUGL	.2207 N(479) **SIG .000**	.0521 N(479) SIG .255	-.0656 N(479) SIG .152	.3003 N(479) **SIG .000**	.0611 N(479) SIG .182	.2528 N(479) **SIG .000**				
HFLAG	.0374 N(479) SIG .414	.5486 N(479) **SIG .000**	.2350 N(479) **SIG .000**	-.0298 N(479) SIG .516	.0479 N(479) SIG .296	-.0943 N(479) **SIG .039**	-.1438 N(479) **SIG .002**			
HCIL	.0650 N(479) SIG .155	.4642 N(479) **SIG .000**	.1867 N(479) **SIG .000**	.0306 N(479) SIG .504	.0787 N(479) SIG .085	-.0702 N(479) SIG .125	-.0414 N(479) SIG .366	**.6944** **N(479)** **SIG .000**		
ROTIF	-.0272 N(479) SIG .553	.2096 N(479) **SIG .000**	.1596 N(479) **SIG .000**	-.0626 N(479) SIG .171	.1101 N(479) **SIG .016**	-.0902 N(479) **SIG .049**	-.0909 N(479) **SIG .047**	.4341 N(479) **SIG .000**	.5500 N(479) **SIG .000**	
TS	-.0613 N(479) SIG .181	-.2351 N(479) **SIG .000**	.0337 N(479) SIG .462	.1005 N(479) **SIG .028**	.0450 N(479) SIG .326	.2811 N(479) **SIG .000**	.1887 N(479) **SIG .000**	-.0859 N(479) SIG .060	-.0826 N(479) SIG .071	.0124 N(479) SIG .787
TVS	-.2038 N(478) **SIG .000**	-.3969 N(478) **SIG .000**	-.0090 N(478) SIG .844	-.0685 N(478) SIG .135	-.0180 N(478) SIG .694	.1046 N(478) **SIG .022**	.0096 N(478) SIG .834	-.1658 N(478) **SIG .000**	-.0519 N(478) SIG .257	.0623 N(478) SIG .174
AFDW	.0782 N(478) SIG .088	-.0793 N(478) SIG .083	.0216 N(478) SIG .638	.1605 N(478) **SIG .000**	.0432 N(478) SIG .346	.2521 N(478) **SIG .000**	.2559 N(478) **SIG .000**	-.0568 N(478) SIG .215	-.1075 N(478) **SIG .019**	-.0358 N(478) SIG .435
TTC	-.4093 N(455) **SIG .000**	-.7187 **N(455)** **SIG .000**	-.1556 N(455) **SIG .001**	-.2623 N(455) **SIG .000**	-.0487 N(455) SIG .300	.0182 N(455) SIG .699	-.2178 N(455) **SIG .000**	-.4078 N(455) **SIG .000**	-.4157 N(455) **SIG .000**	-.1265 N(455) **SIG .007**
CHLO	.1467 N(474) **SIG .001**	.2615 N(474) **SIG .000**	.0870 N(474) SIG .058	.1821 N(474) **SIG .000**	.0268 N(474) SIG .561	.0190 N(474) SIG .679	.3694 N(474) **SIG .000**	.1332 N(474) **SIG .004**	.1998 N(474) **SIG .000**	.0221 N(474) SIG .631
TOC	-.4140 N(434) **SIG .000**	-.1393 N(434) **SIG .004**	-.1288 N(434) **SIG .007**	-.2485 N(434) **SIG .000**	.0114 N(434) SIG .813	-.0247 N(434) SIG .607	-.2185 N(434) **SIG .000**	.1983 N(434) **SIG .000**	.1479 N(434) **SIG .002**	.1344 N(434) **SIG .005**

	TS	TVS	AFDW	TTC	CHLO
TVS	.6153 **N(503)** **SIG .000**				
AFDW	.9105 **N(503)** **SIG .000**	.3209 N(502) **SIG .000**			
TTC	.0750 N(479) SIG .101	.2567 N(478) **SIG .000**	-.0522 N(478) SIG .255		
CHLO	-.0006 N(499) SIG .989	-.0503 N(498) SIG .263	.0378 N(498) SIG .399	-.4747 N(474) **SIG .000**	
TOC	-.1978 N(434) **SIG .000**	.0108 N(433) SIG .822	-.2751 N(433) **SIG .000**	.2651 N(419) **SIG .000**	-.1315 N(429) **SIG .006**

Table 4. Phytoplankton and Water Quality Variables Significantly Correlated With Dose.

	Spearman's rho	N	SIG
pH	-.1762	192	0.014
Chlamydomonas	.1589	191	0.028
Nostoc	-.1534	192	0.034
Euglenoids	.1595	192	0.027
TTC Respiration	-.1477	192	0.041
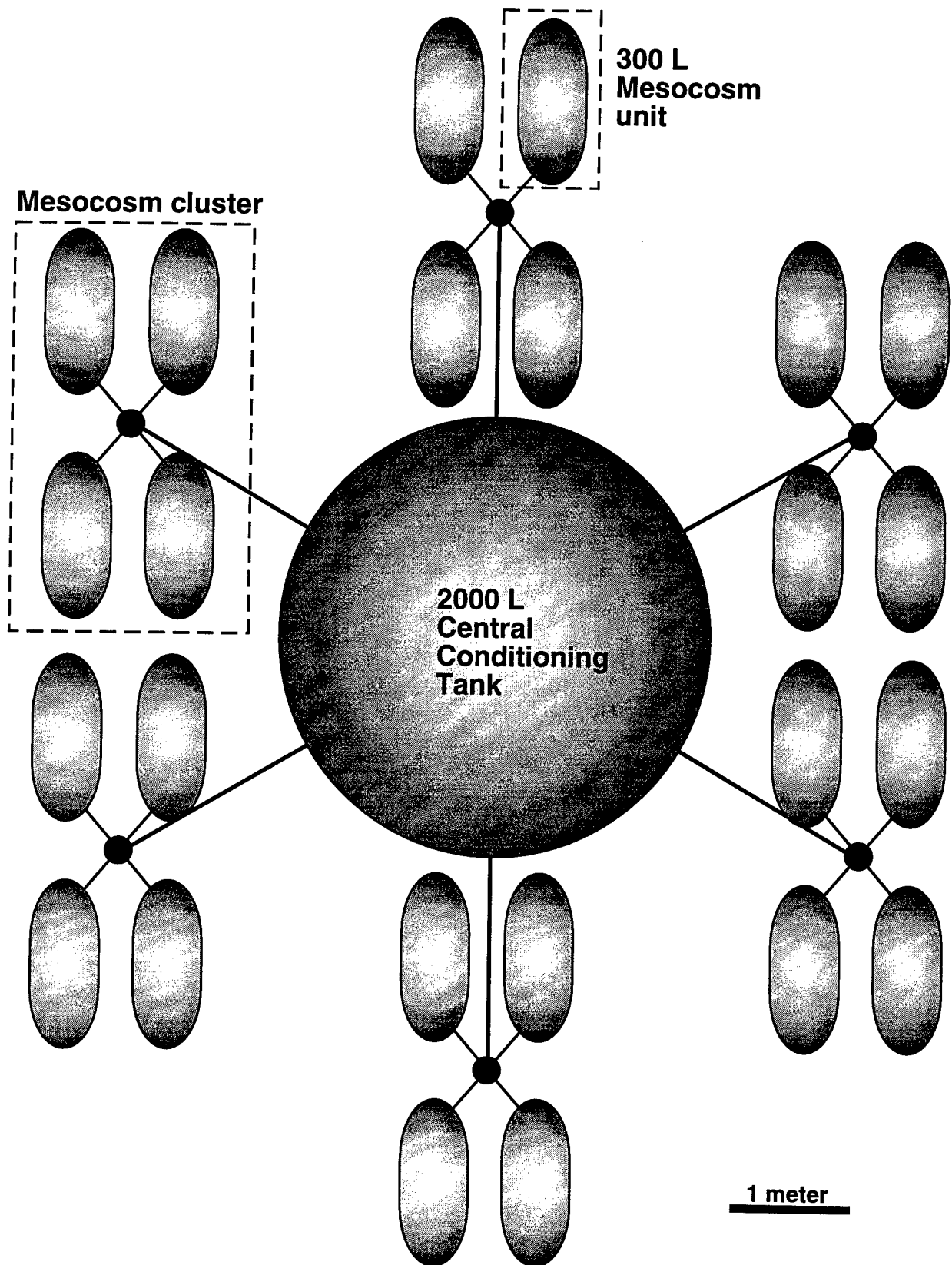
Figure 1. Design for the outdoor mesocosms at the IETC research site.
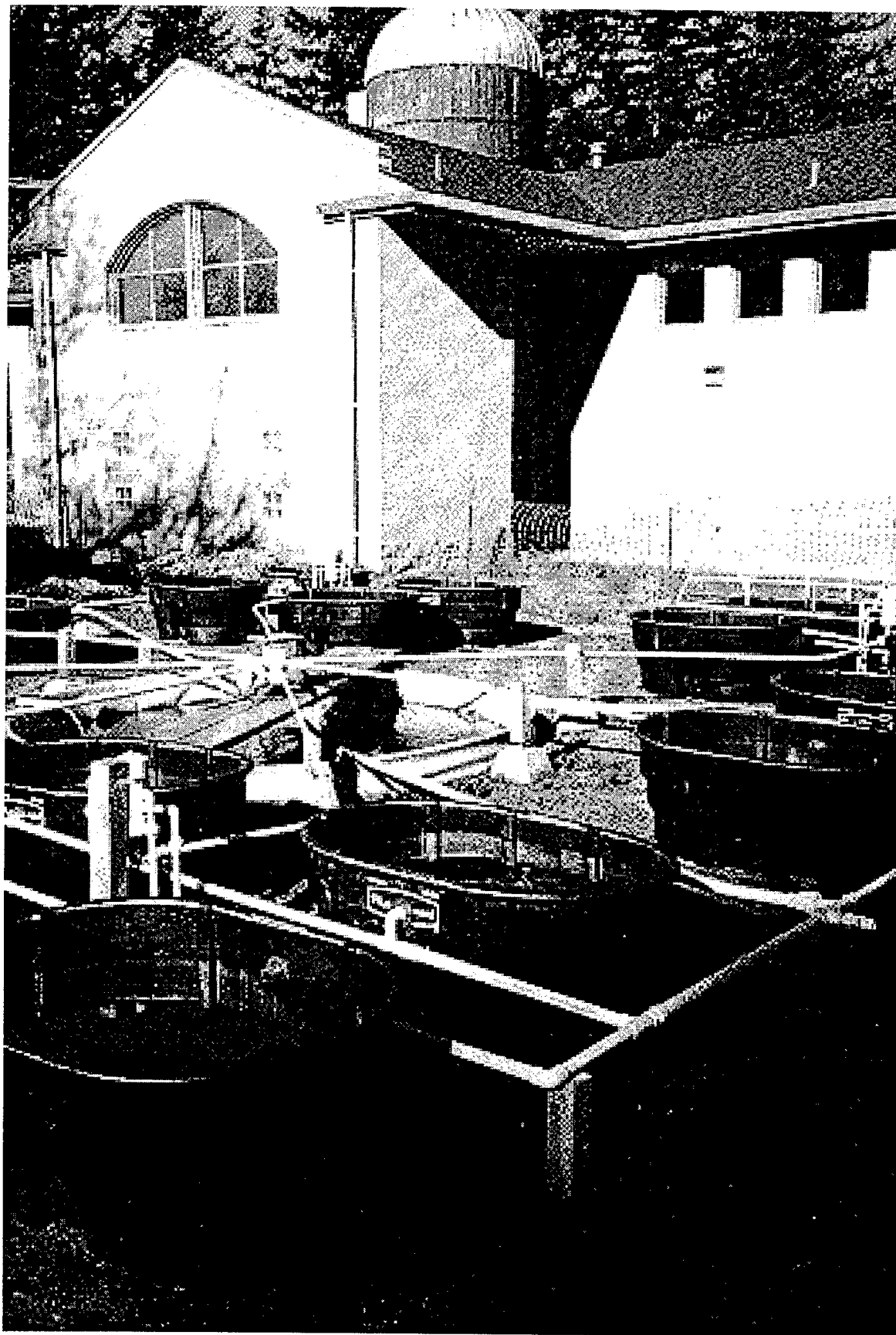
Figure 2. Photograph of uncovered mesocosms at the IETC research site.
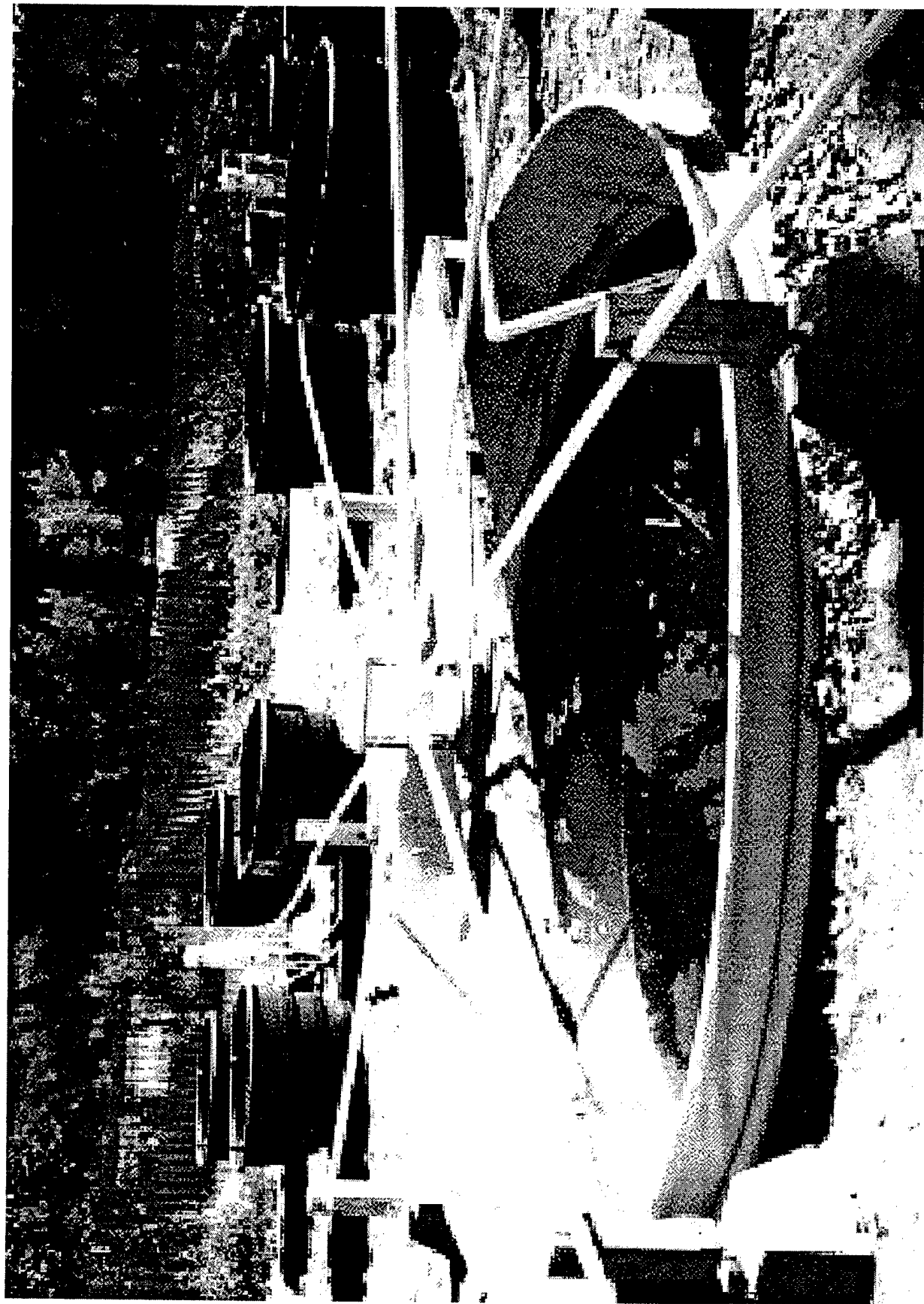
Figure 3. Photograph of central conditioning tank at the IETC research site and mesocosm clusters.

Figure 4. Photograph of covered mesocosms at the IETC research site.

Figure 5a. Periphyton - Ciliates
Algal Grazer (Green), High Dose
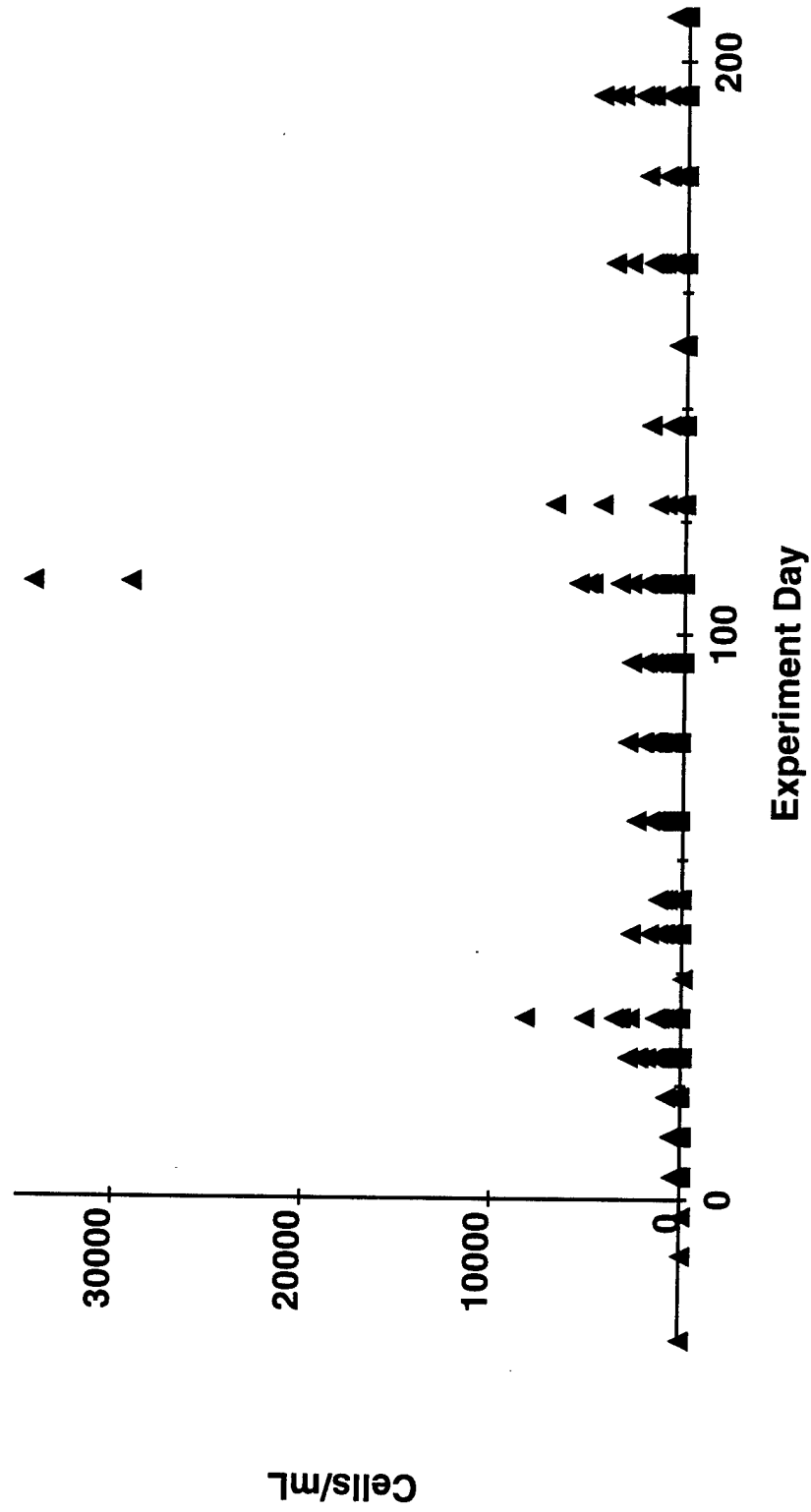
Figure 5b. Periphyton - Ciliates
Algal Grazer (Green) - Mid-Dose
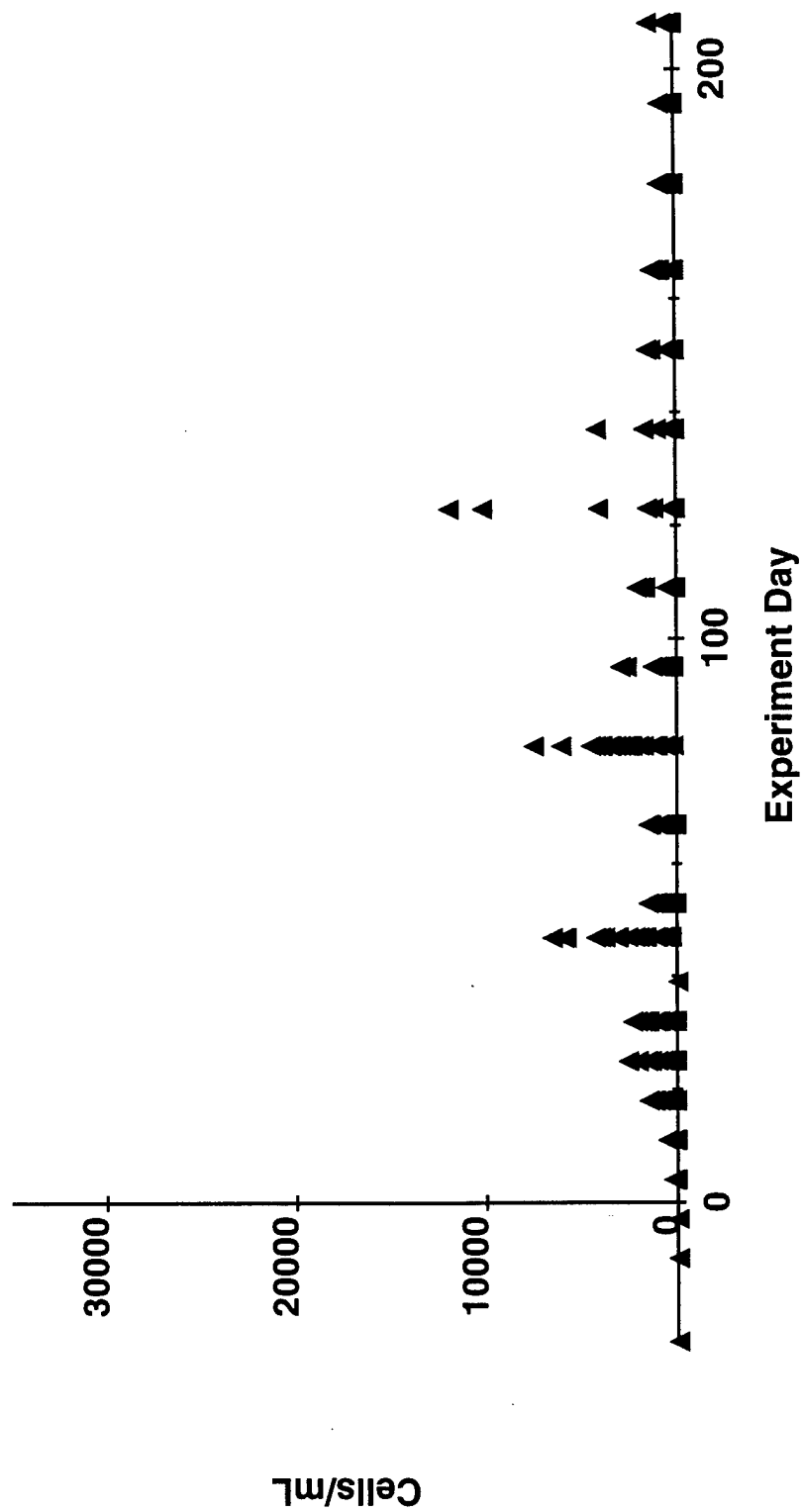
Figure 5c. Periphyton - Ciliates
Algal Grazer (Green) - Low Dose
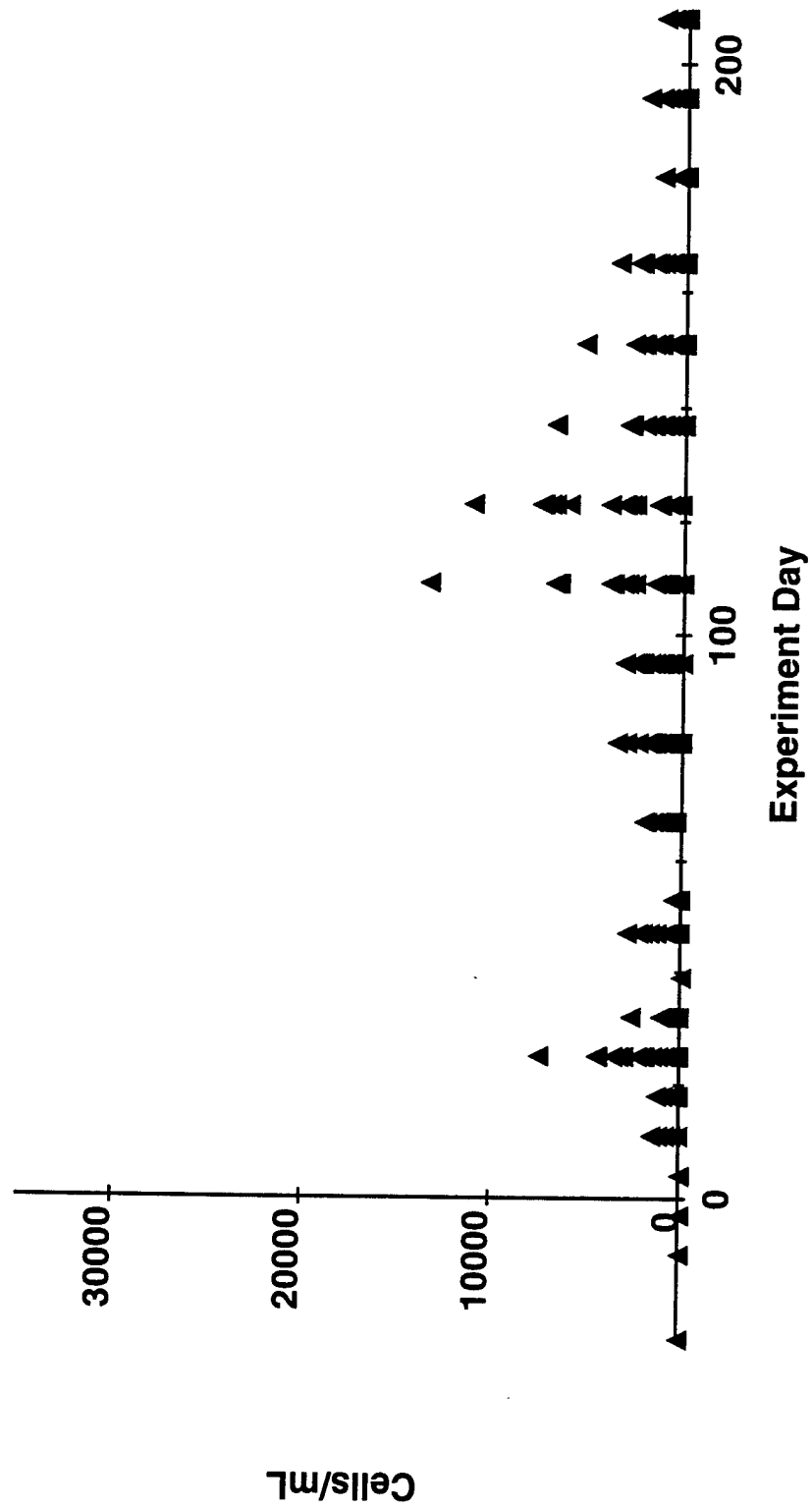
Figure 5d. Periphyton - Ciliates
Algal Grazer (Green) - Undosed
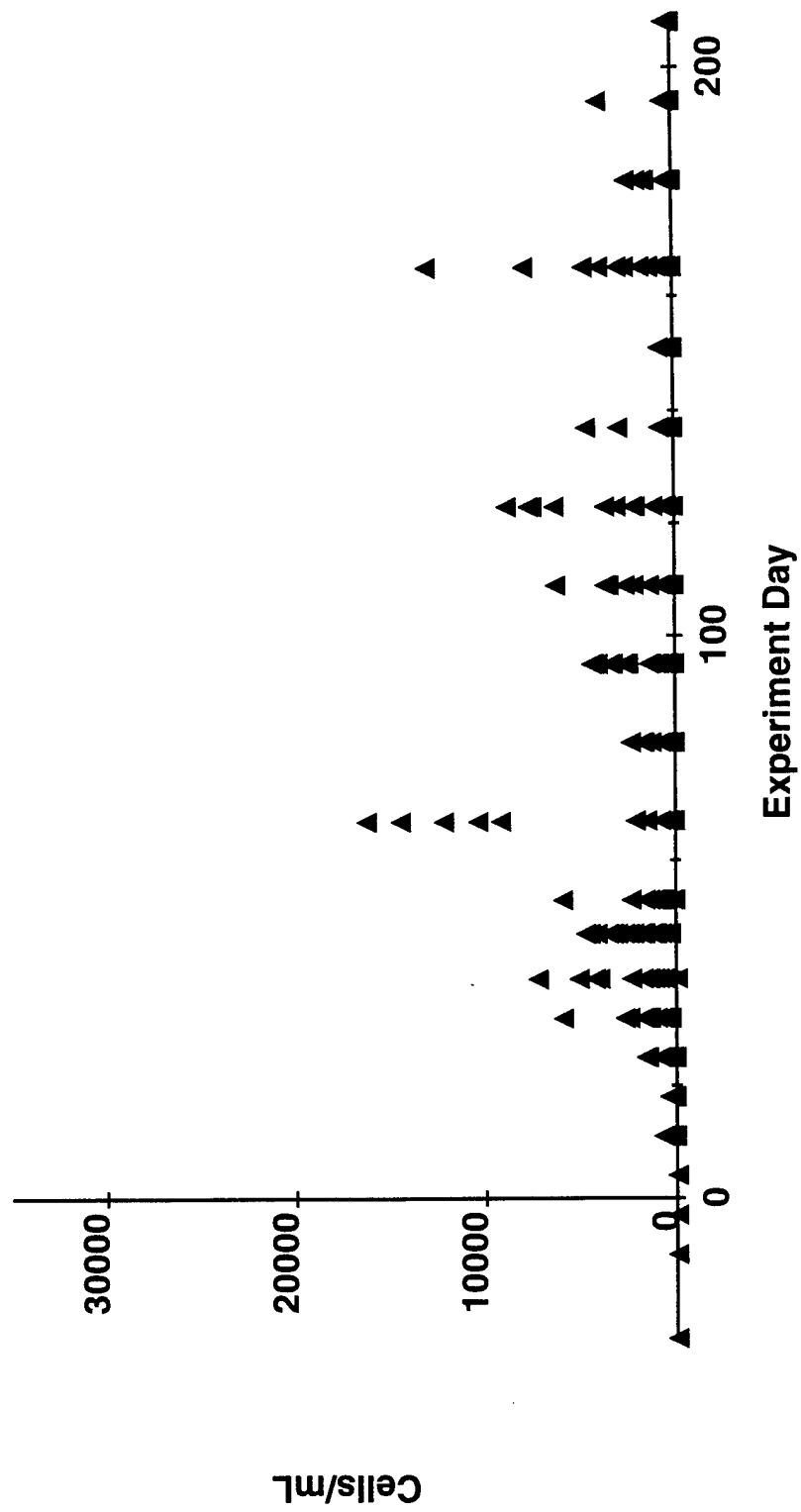
Figure 6a. Periphyton - Ciliates
Other, High Dose
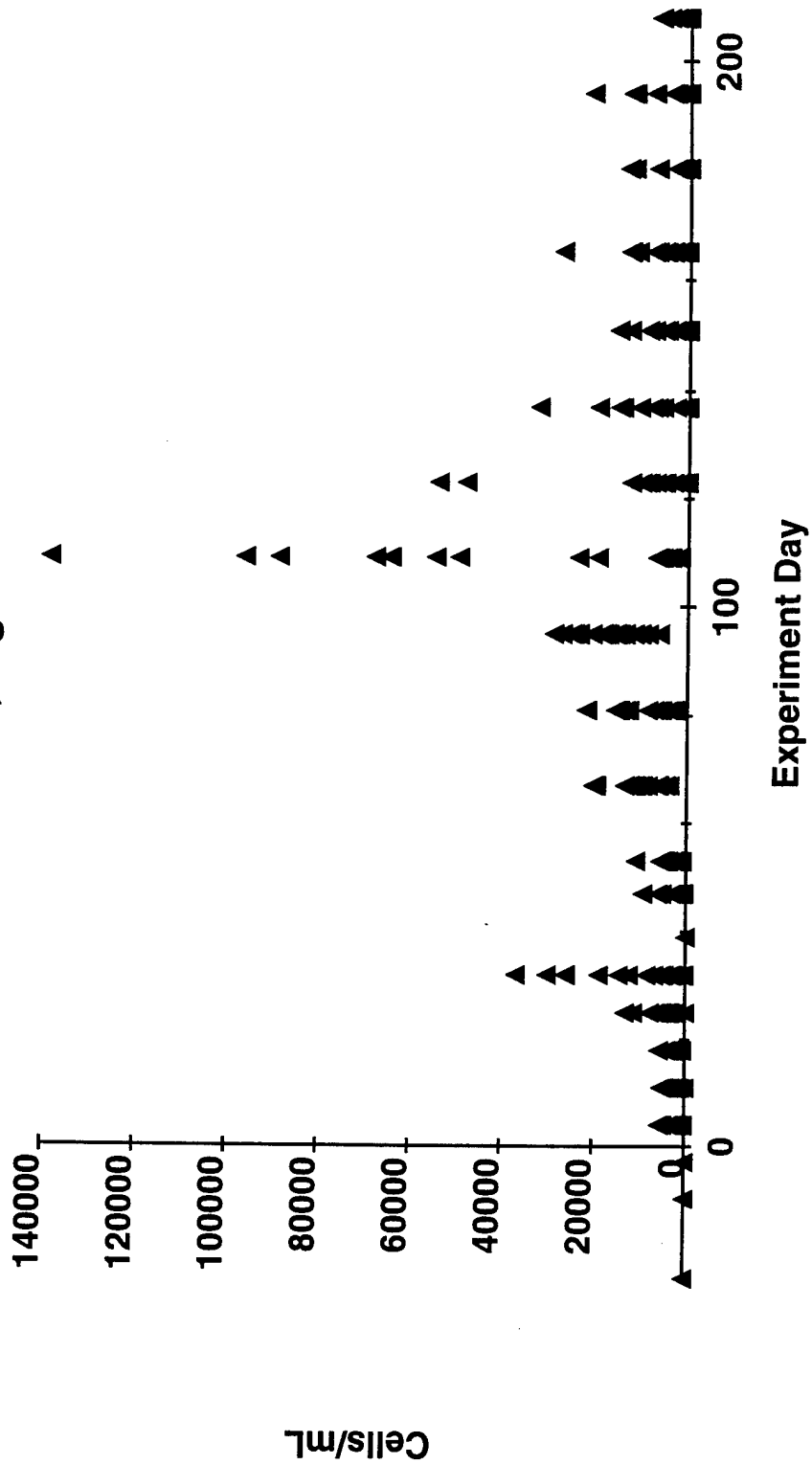
Figure 6b. Periphyton - Ciliates
Other - Mid-Dose
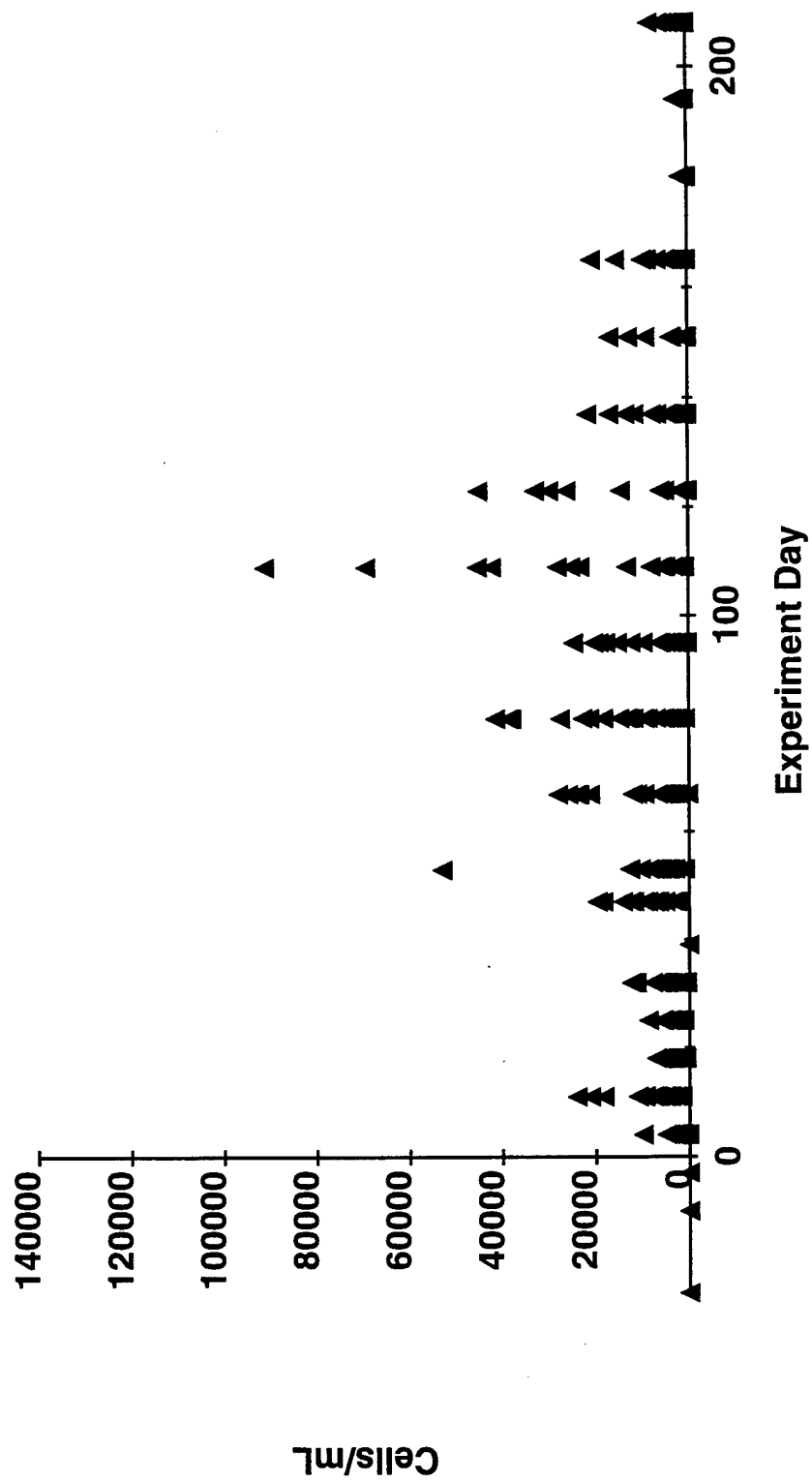
Figure 6c. Periphyton - Ciliates
Other - Low Dose
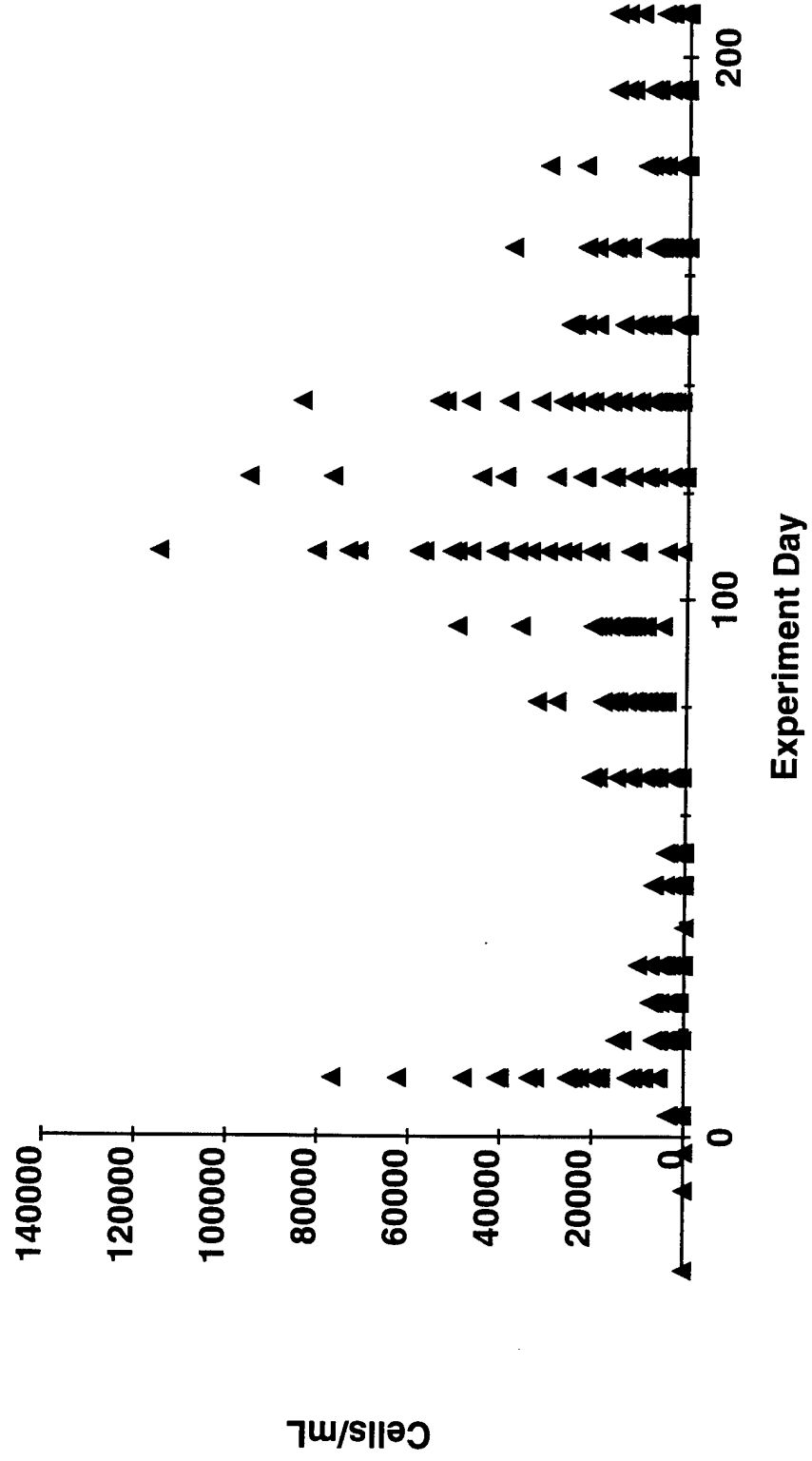
Figure 6d. Periphyton - Ciliates
Other - Undosed
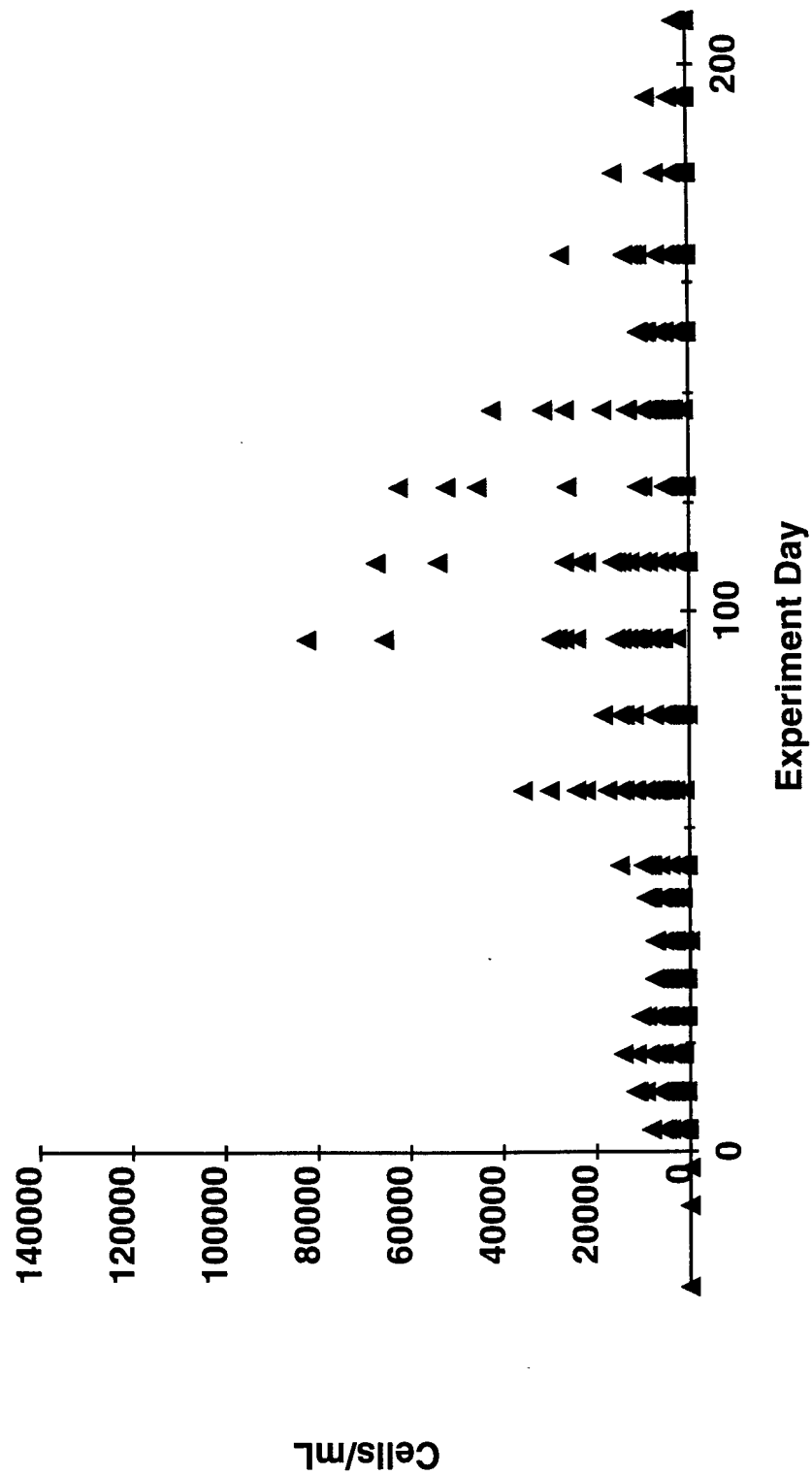
Figure 7a. Periphyton - Cyanophytes
Nostoc, High Dose
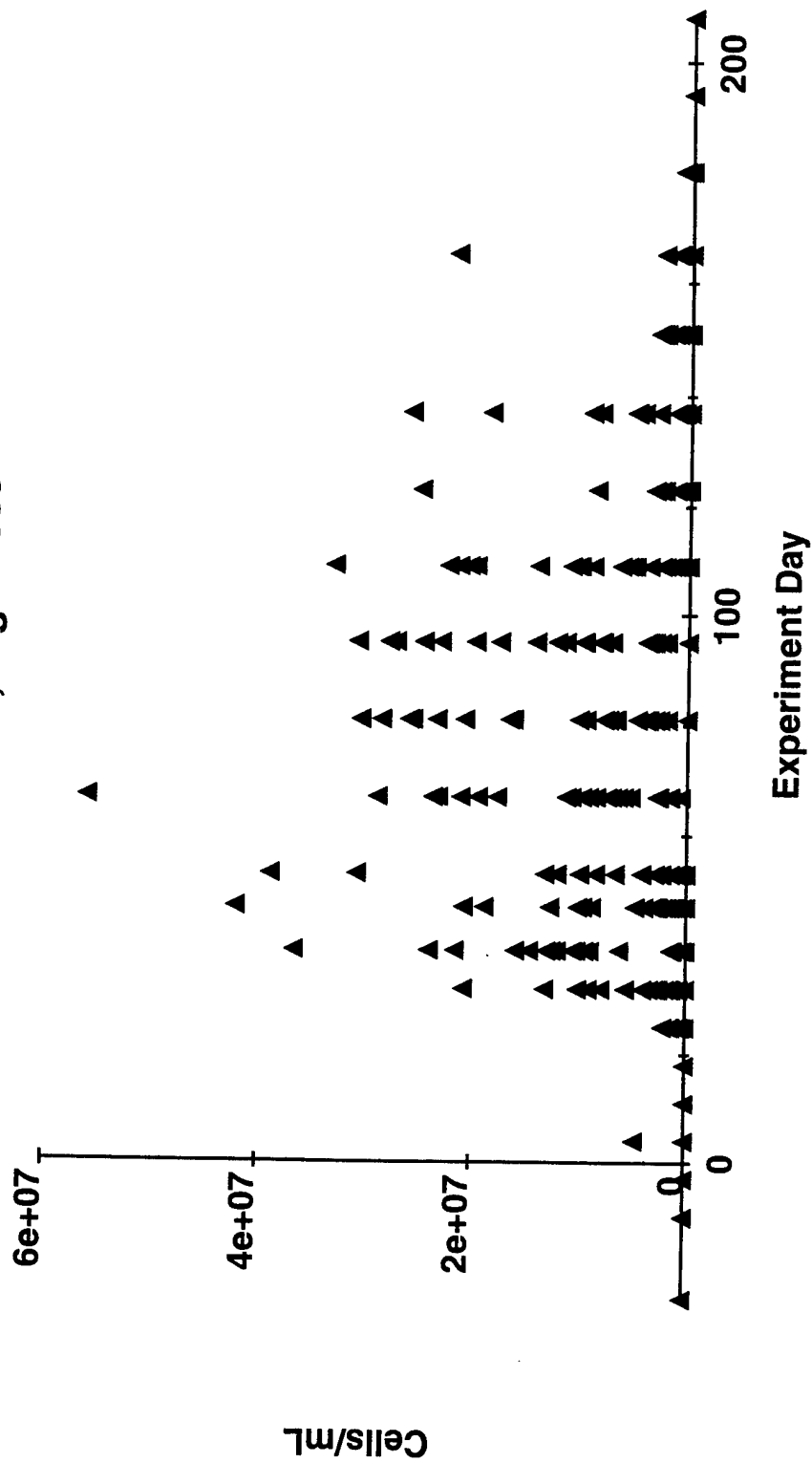
Figure 7b. Periphyton - Cyanophytes
Nostoc - Mid-Dose
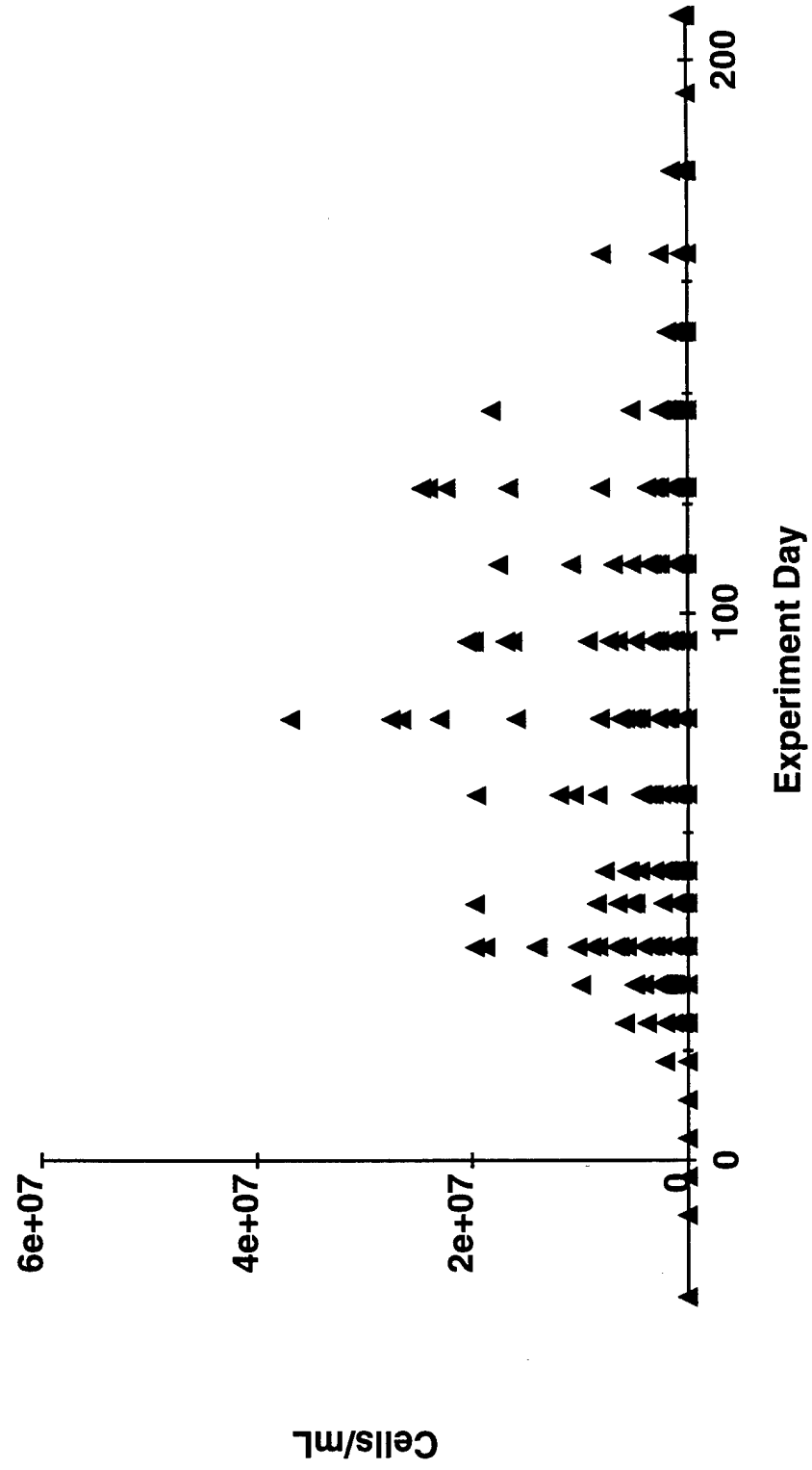
Figure 7c. Periphyton - Cyanophytes
Nostoc - Low Dose
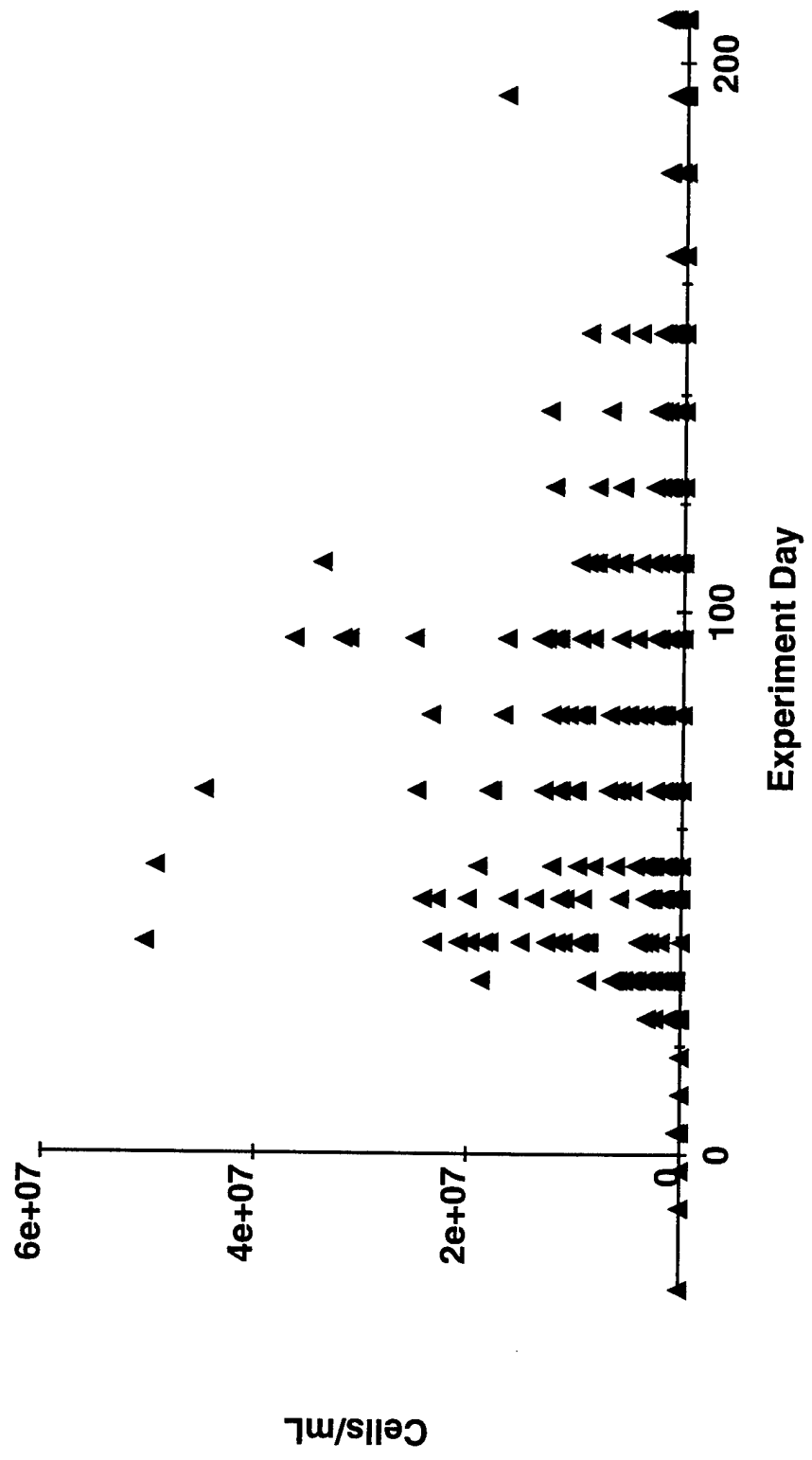
Figure 7d. Periphyton - Cyanophytes
Nostoc - Undosed
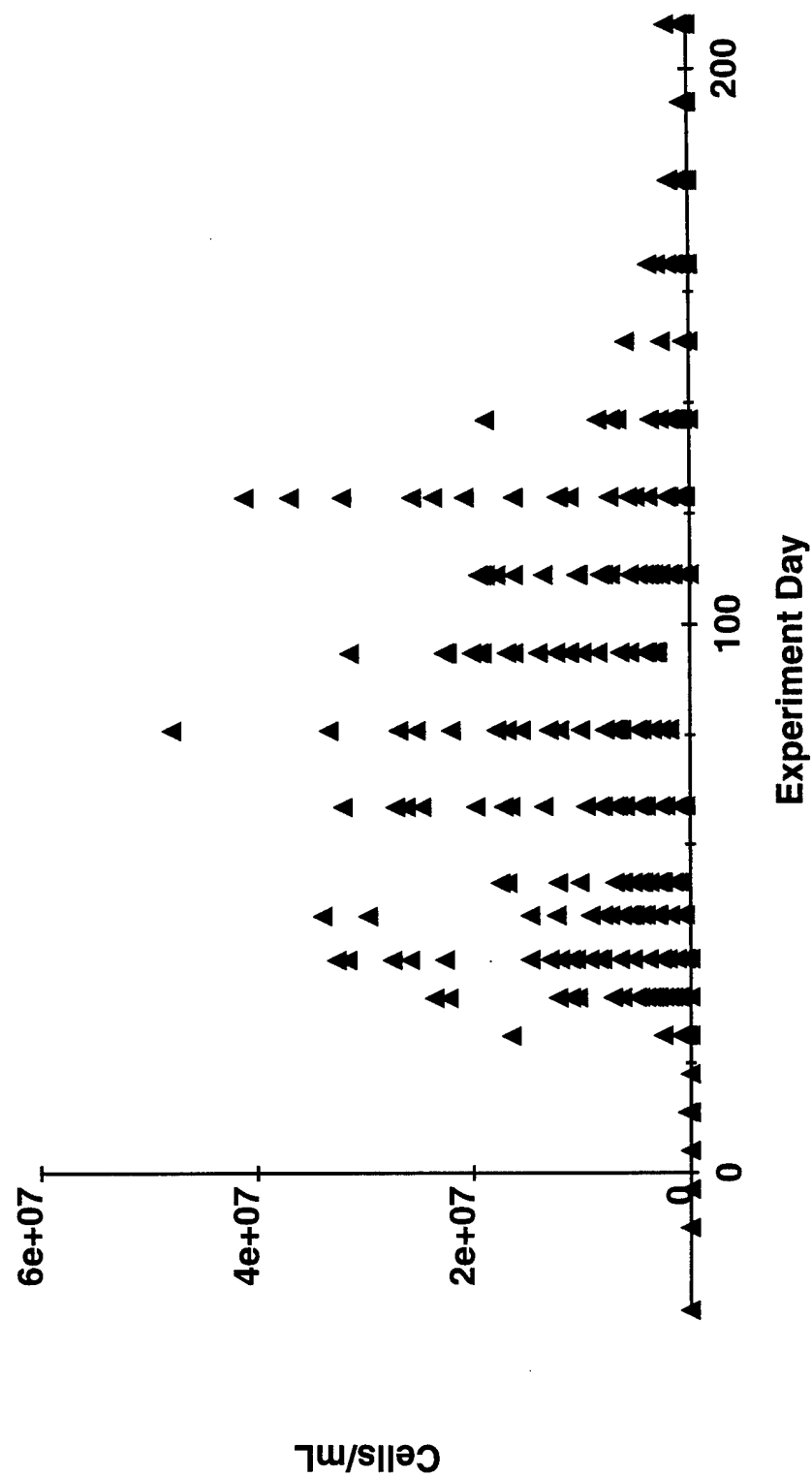
Figure 8a. Periphyton - Cyanophytes
Oscillatoria, High Dose
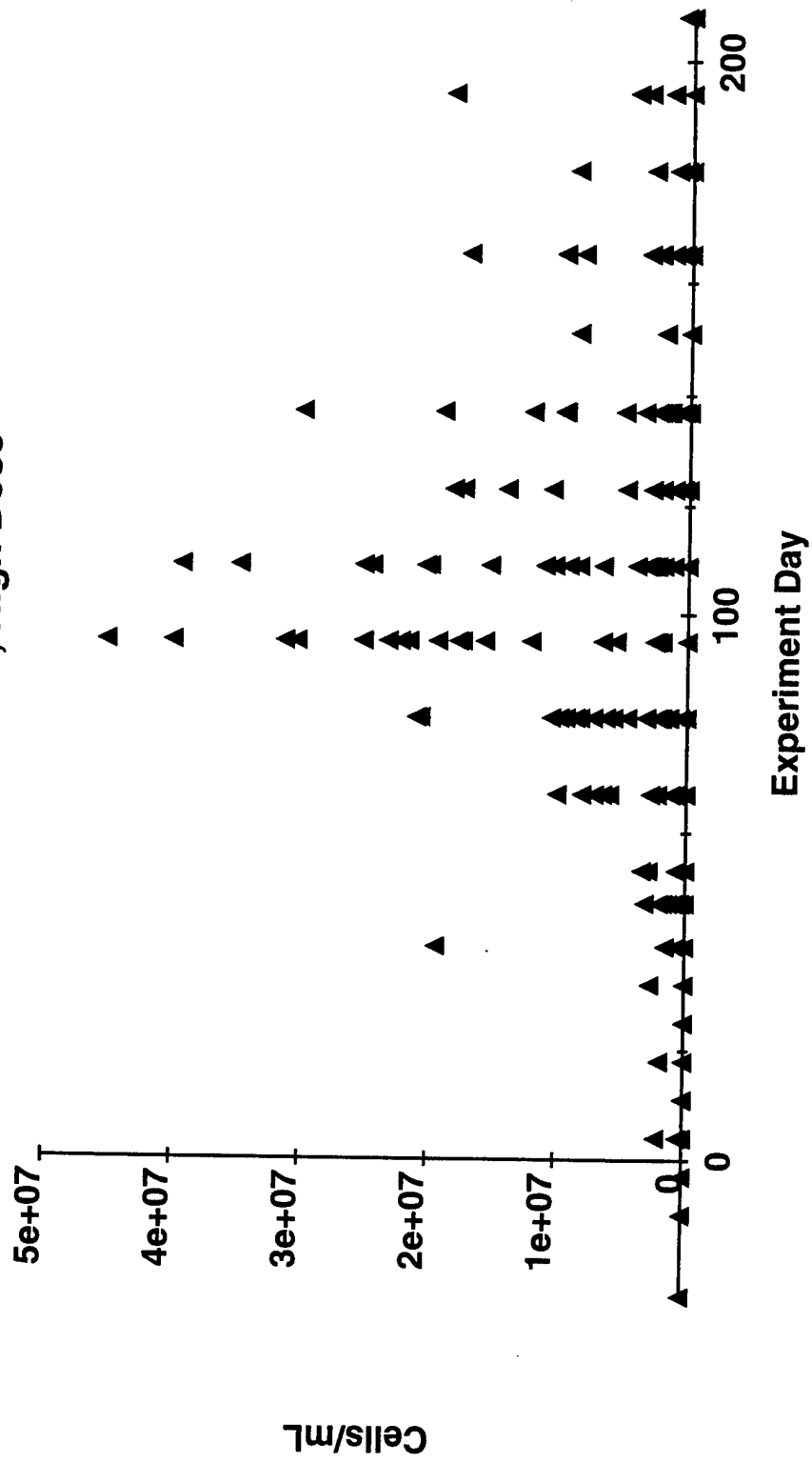
Figure 8b. Periphyton - Cyanophytes
Oscillatoria - Mid-Dose
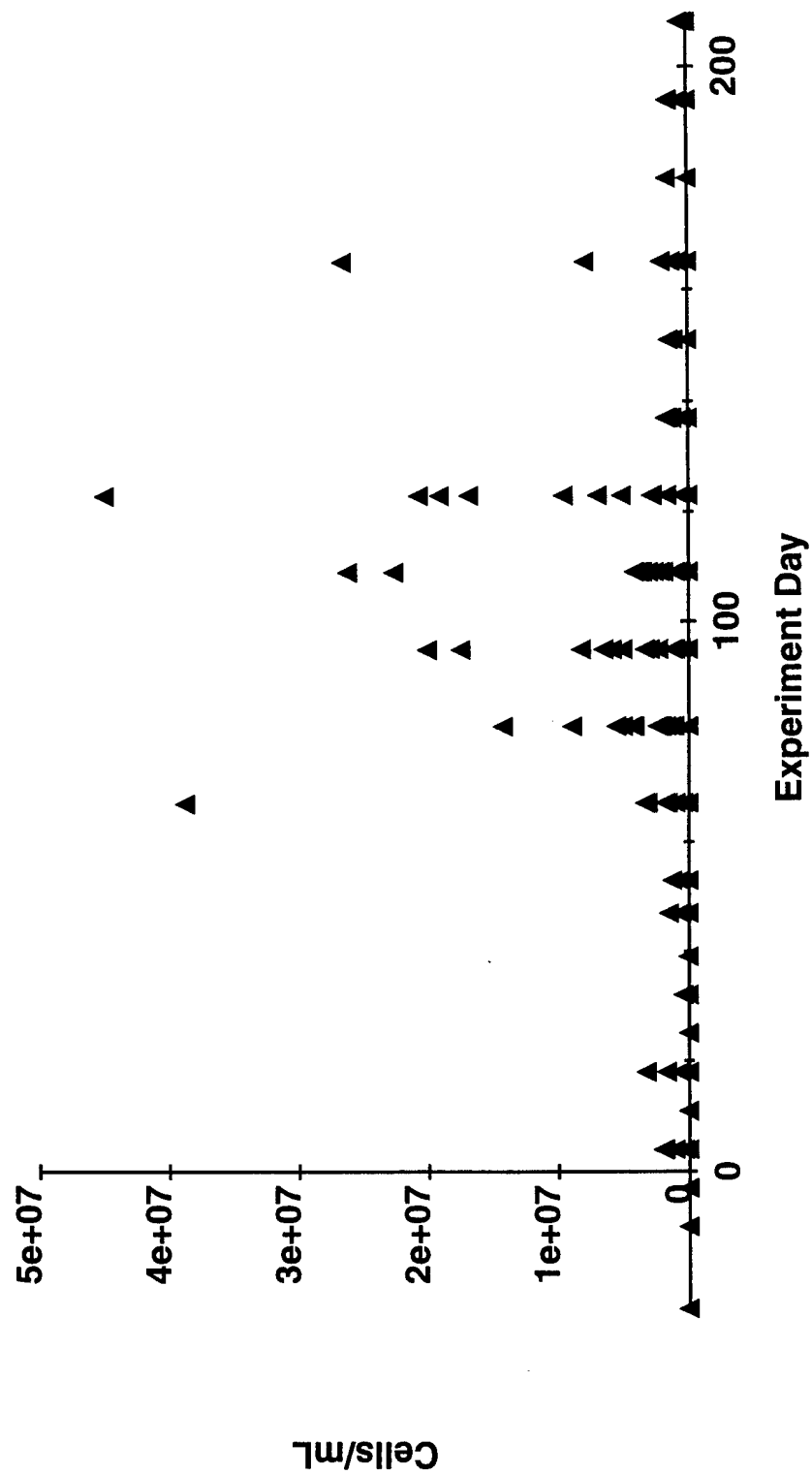
Figure 8c. Periphyton - Cyanophytes
Oscillatoria - Low Dose
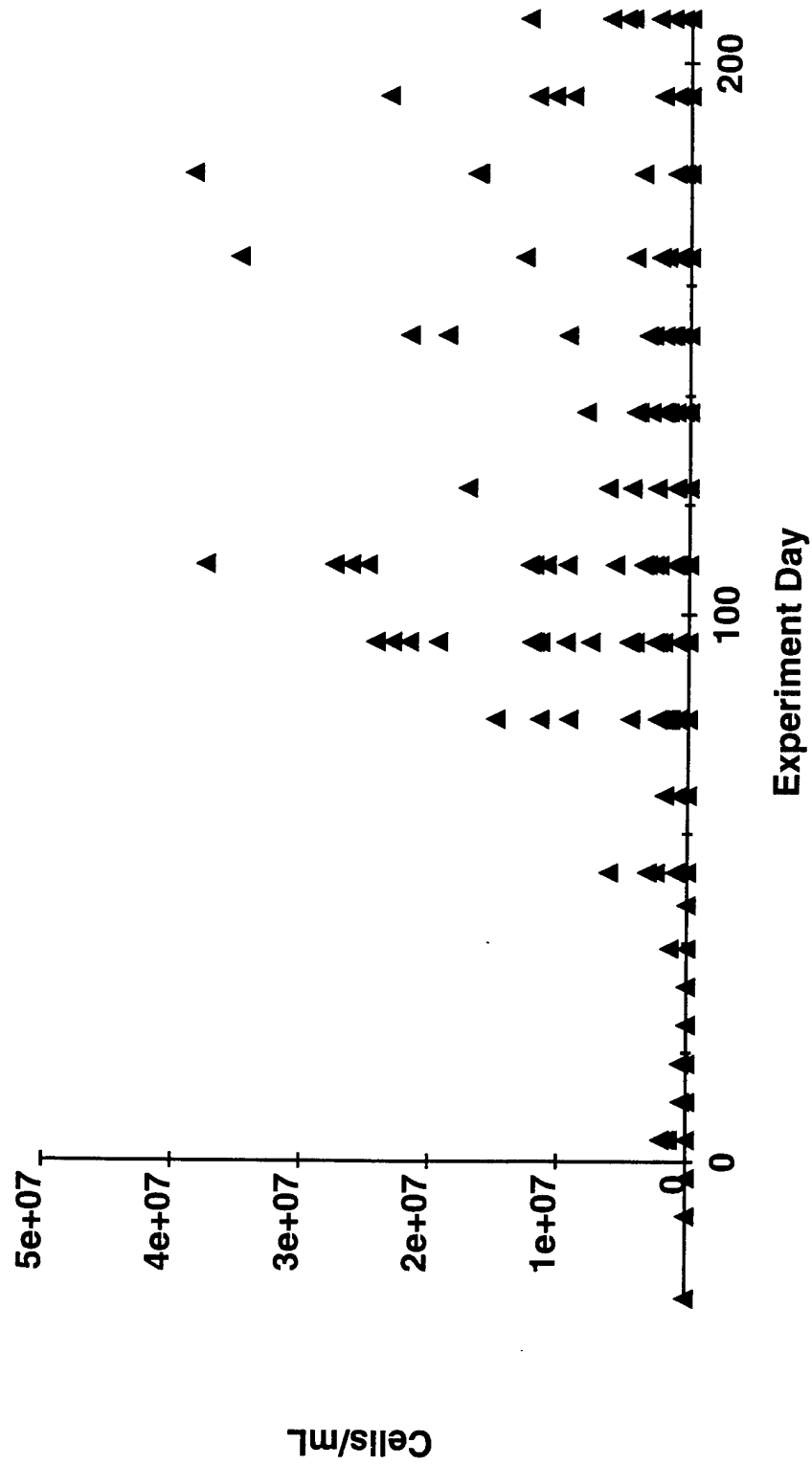
Figure 8d. Periphyton - Cyanophytes
Oscillatoria - Undosed
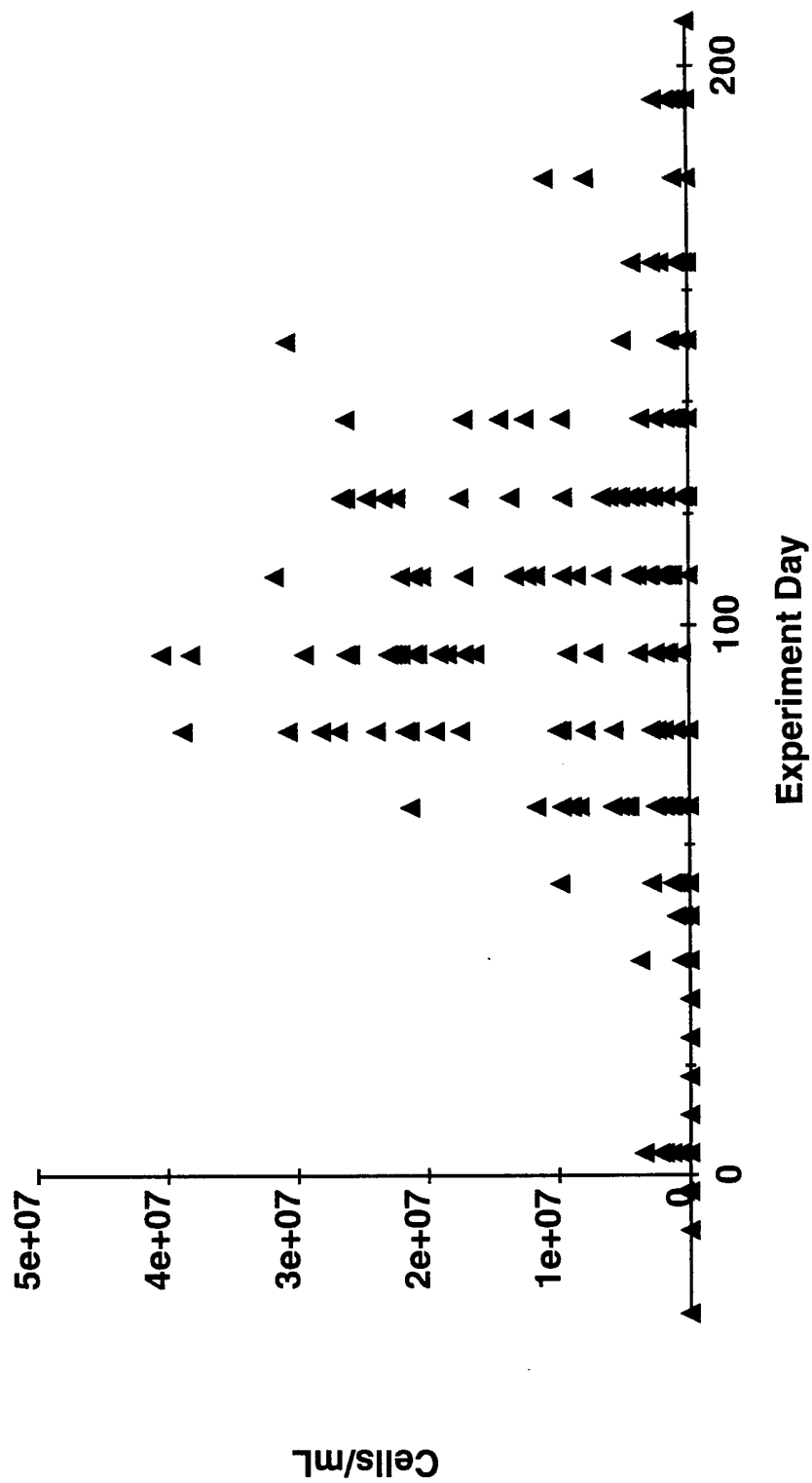
Figure 9a. Periphyton - Diatoms
Centric, High Dose
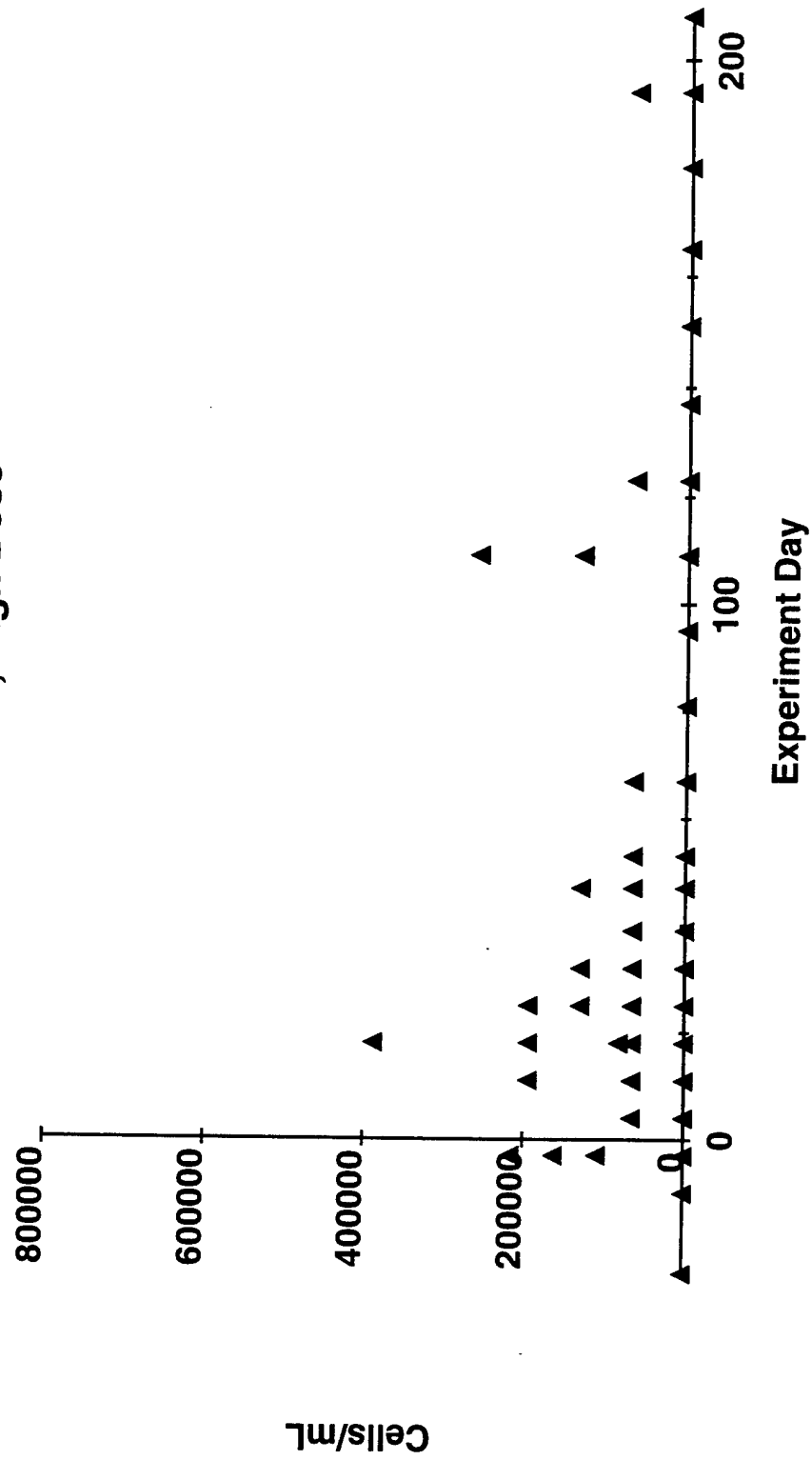
Figure 9b. Periphyton - Diatoms
Centric - Mid-Dose
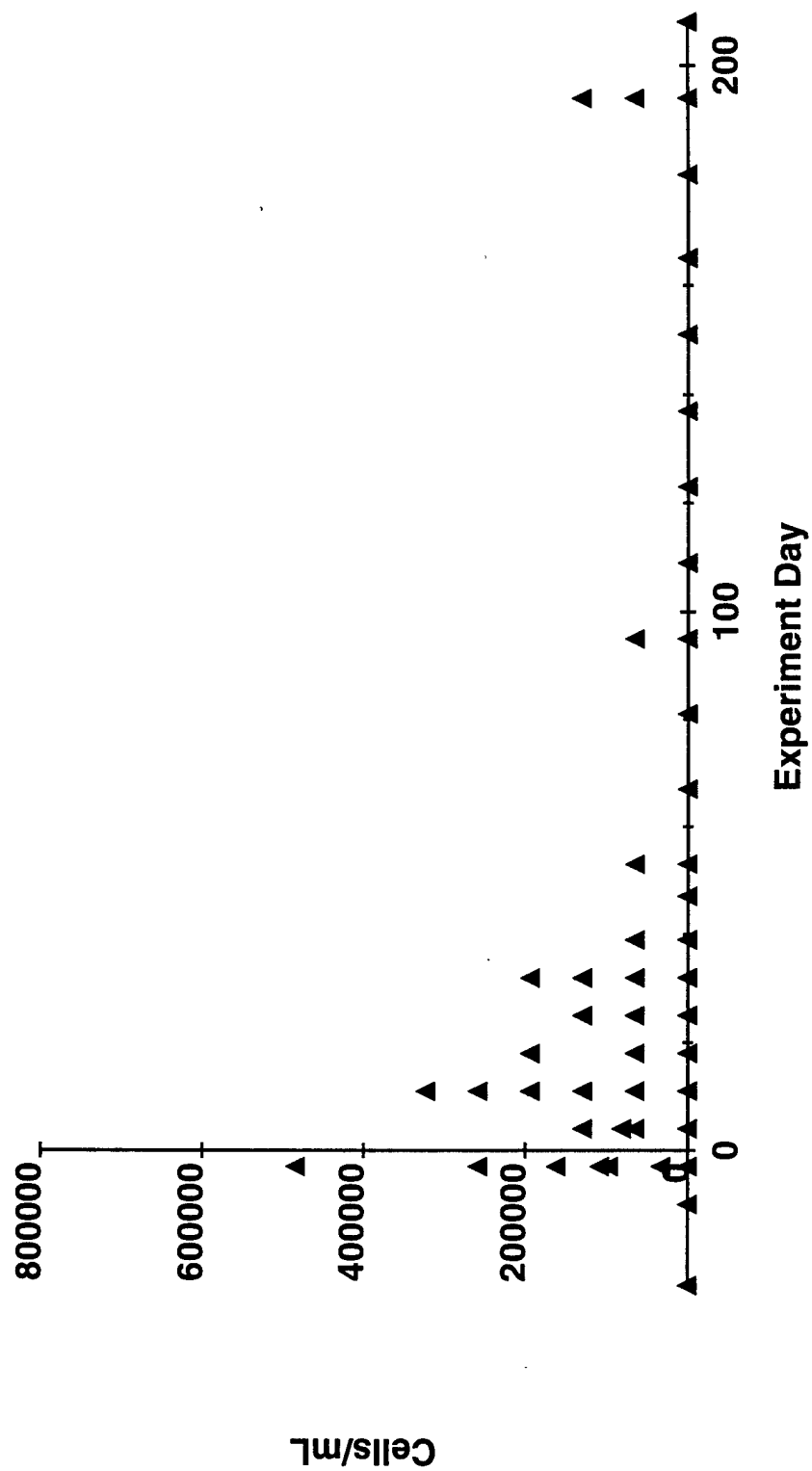
Figure 9c. Periphyton - Diatoms
Centric - Low Dose
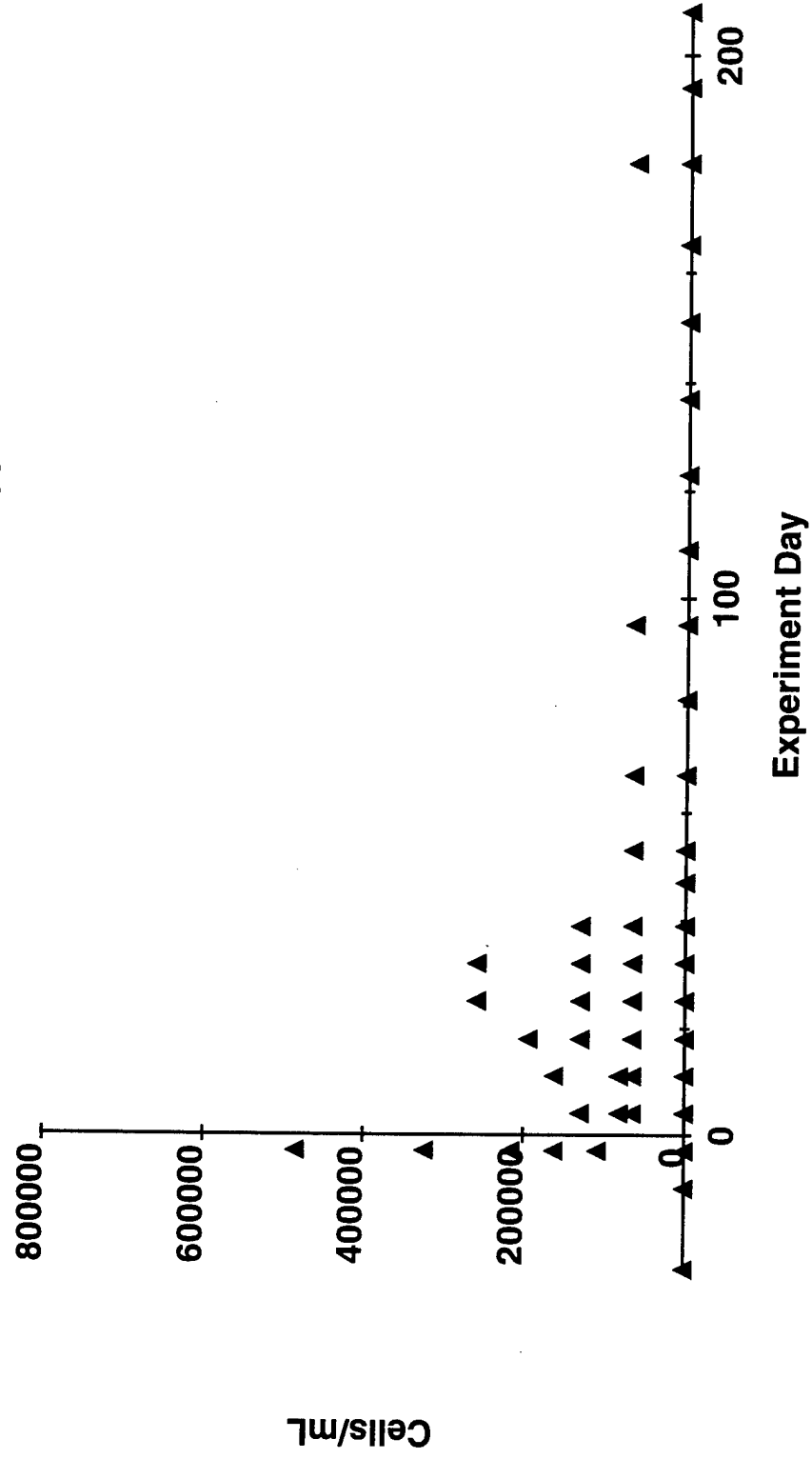
Figure 9d. Periphyton - Diatoms
Centric - Undosed
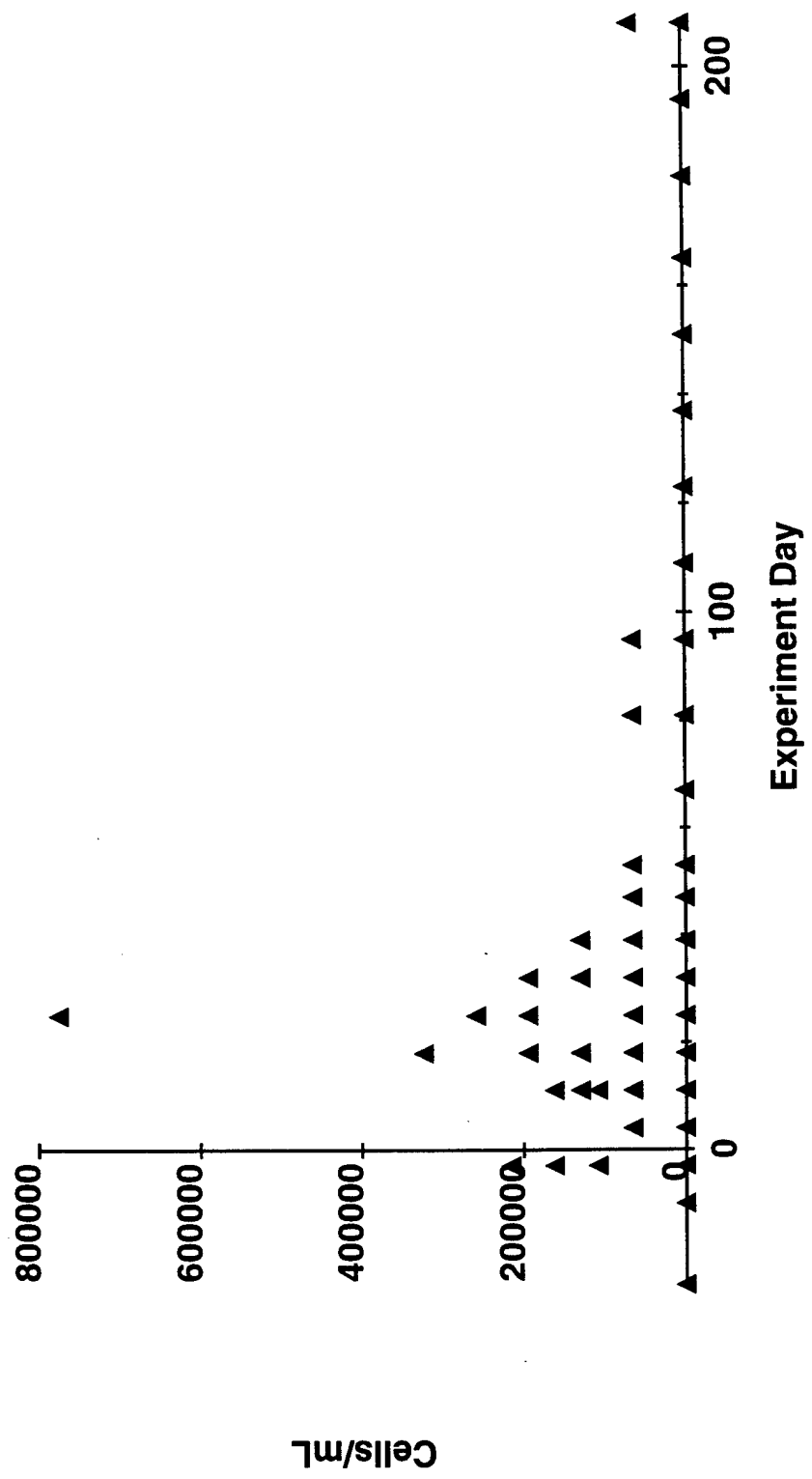
Figure 10a. Periphyton - Diatoms
Melosira, High Dose
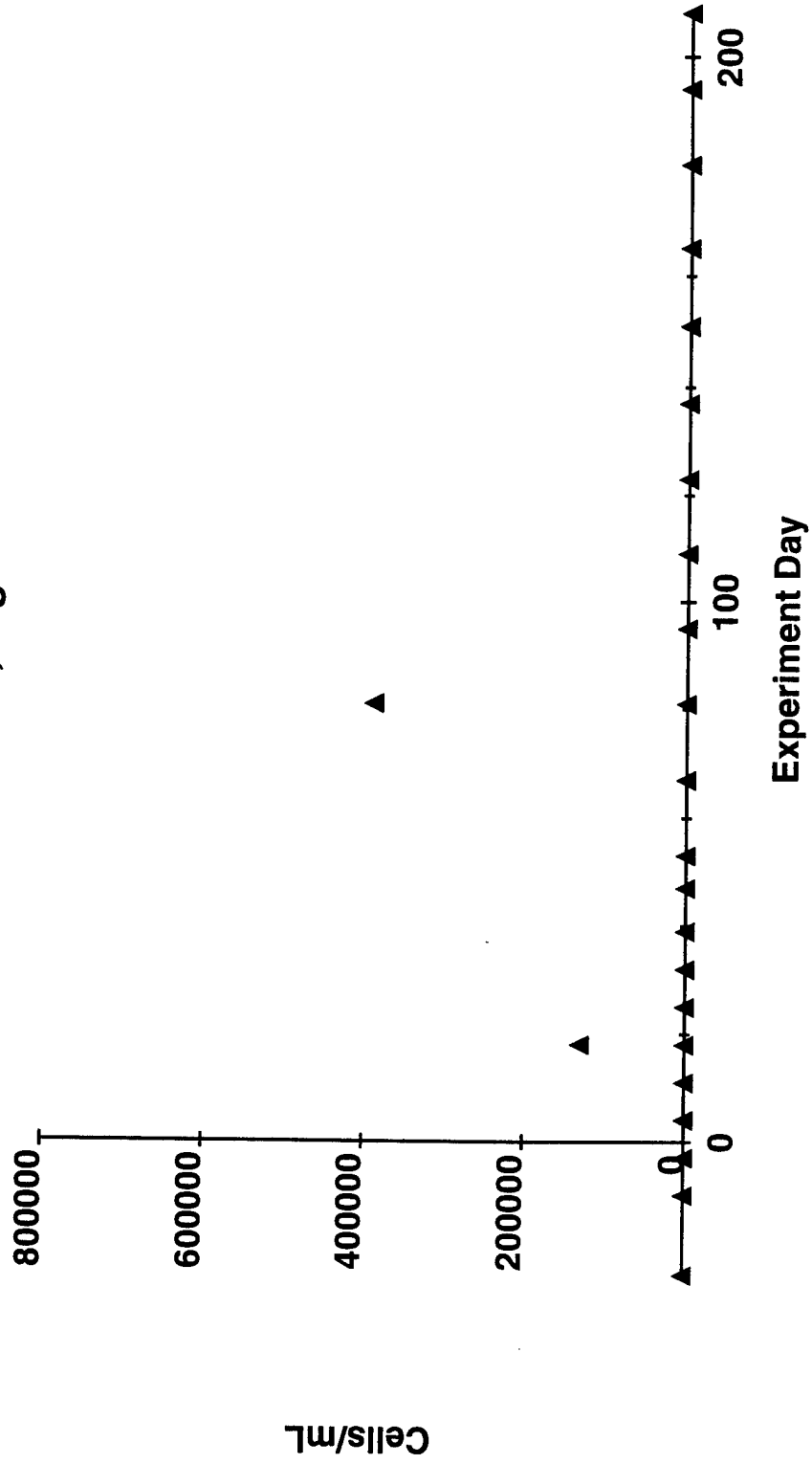
Figure 10b. Periphyton - Diatoms
Melosira - Mid-Dose
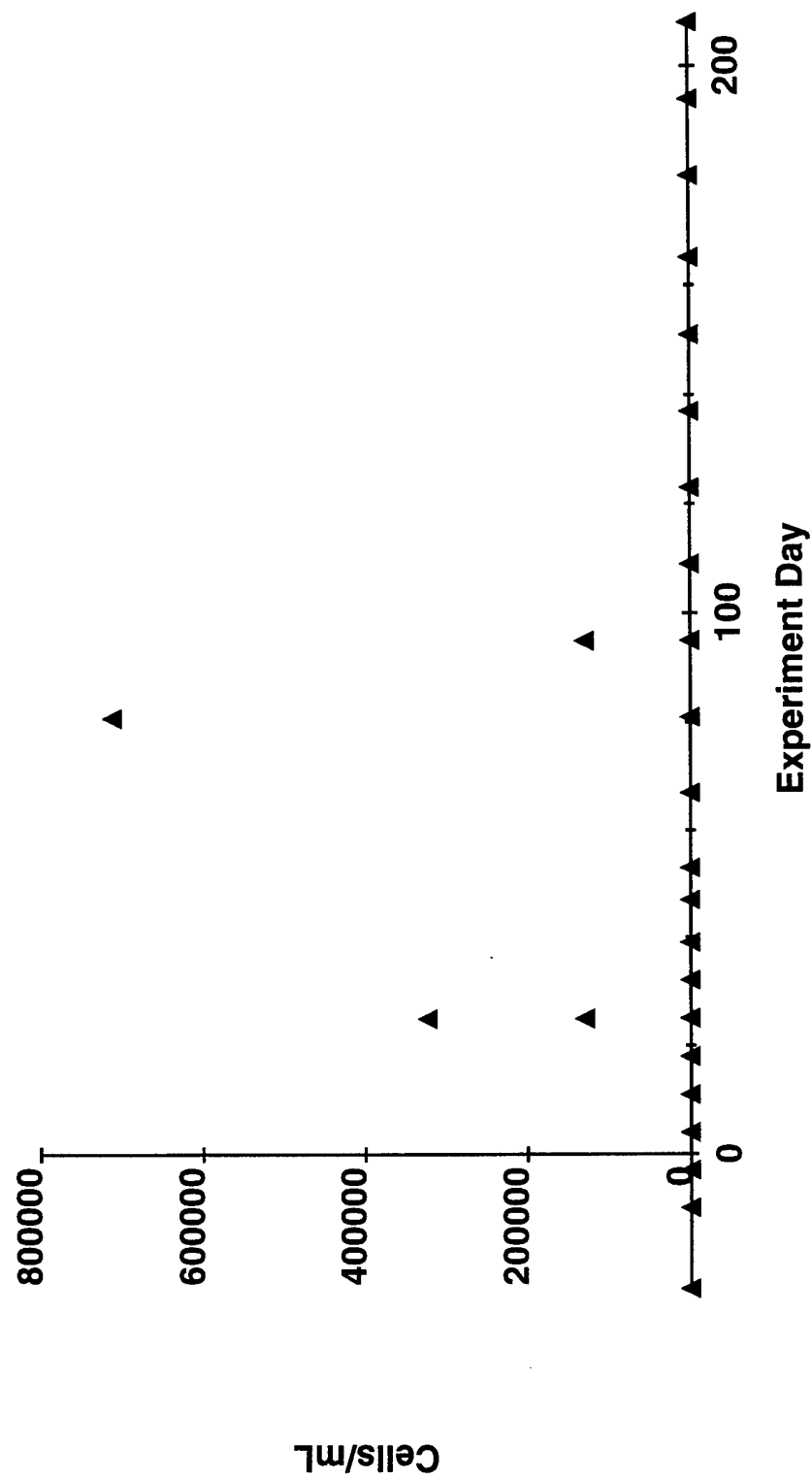
Figure 10c. Periphyton - Diatoms
Melosira - Low Dose

Cells/mL

Experiment Day
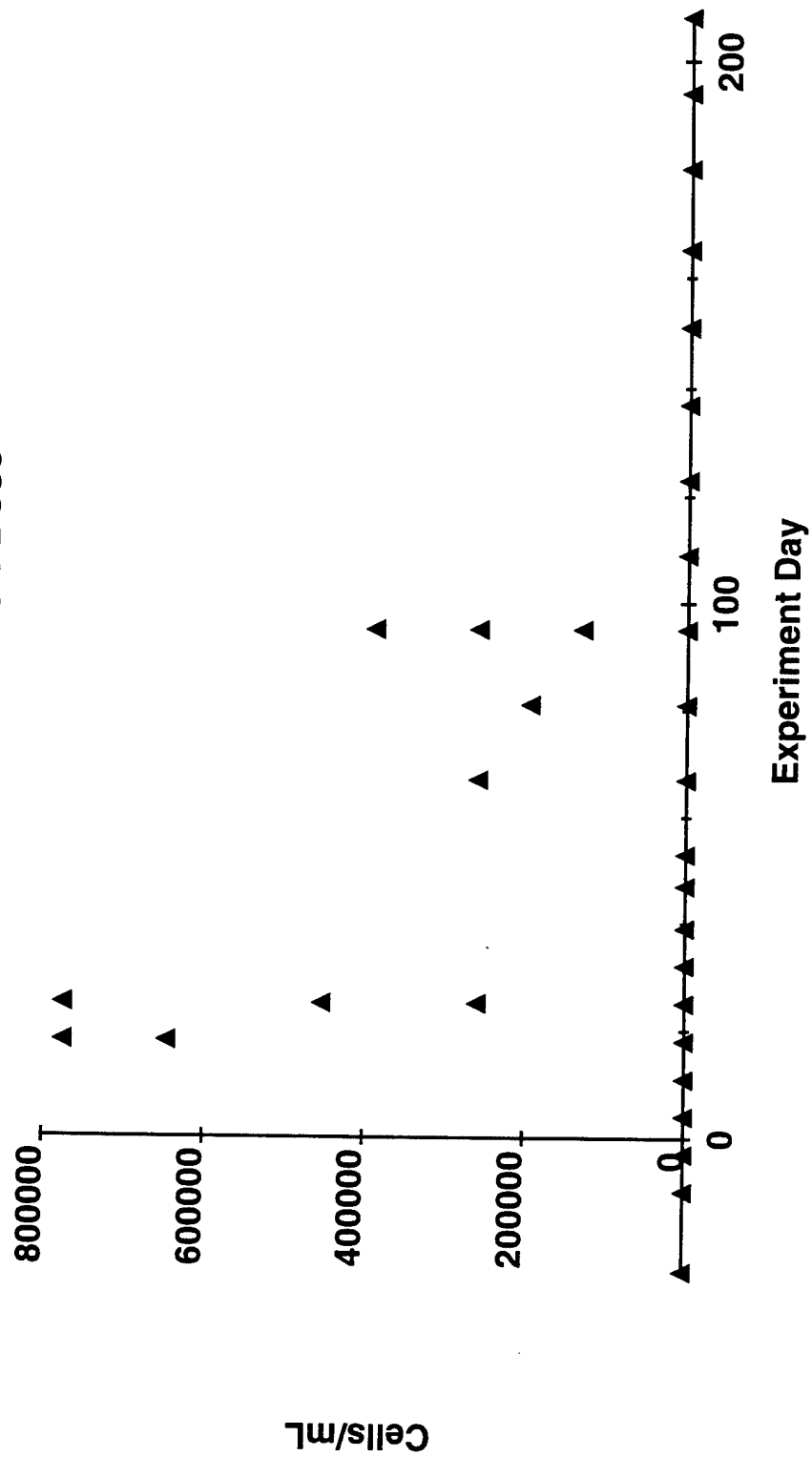
Figure 10d. Periphyton - Diatoms
Melosira - Undosed
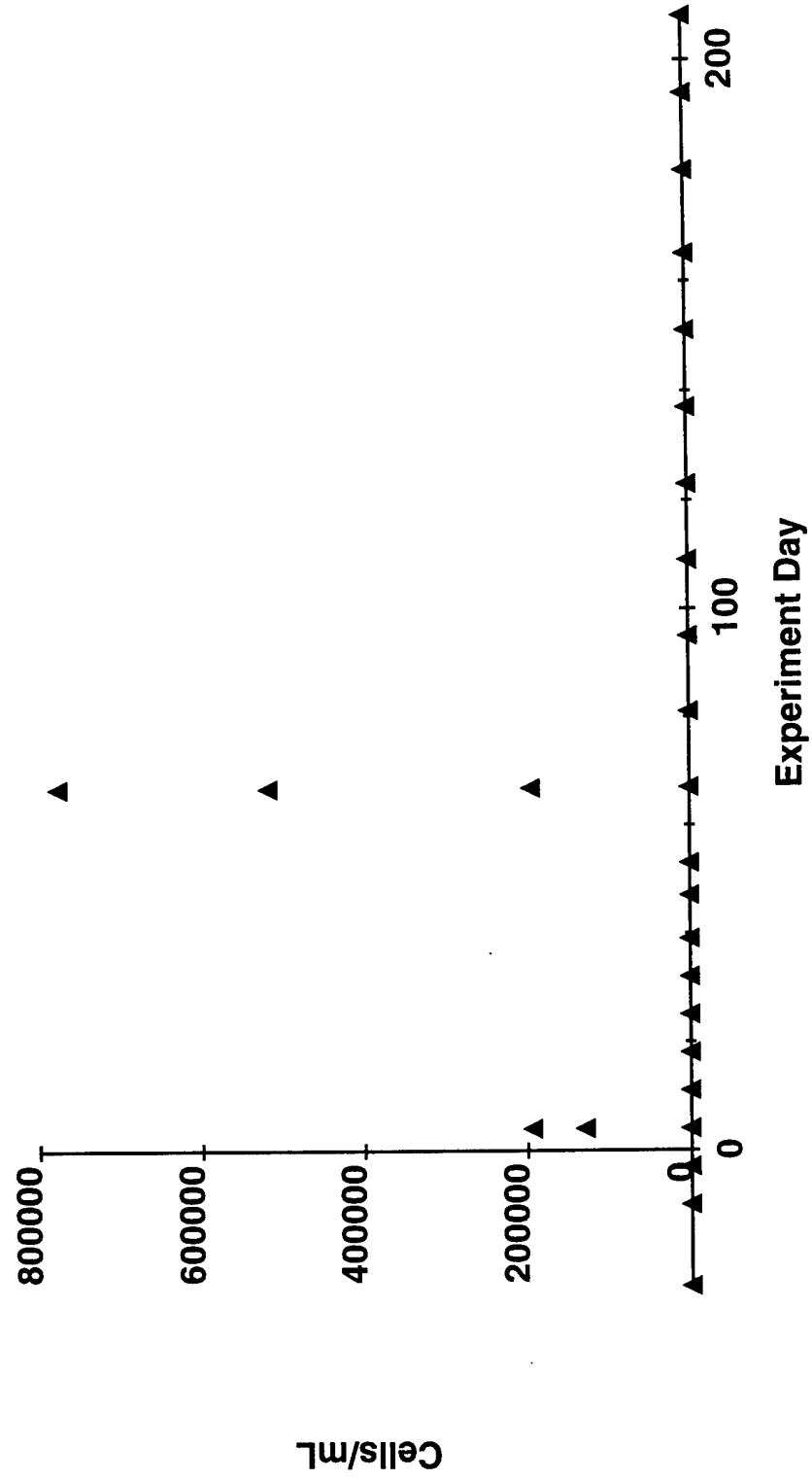
Figure 11a. Periphyton - Diatoms
Pennate, High Dose
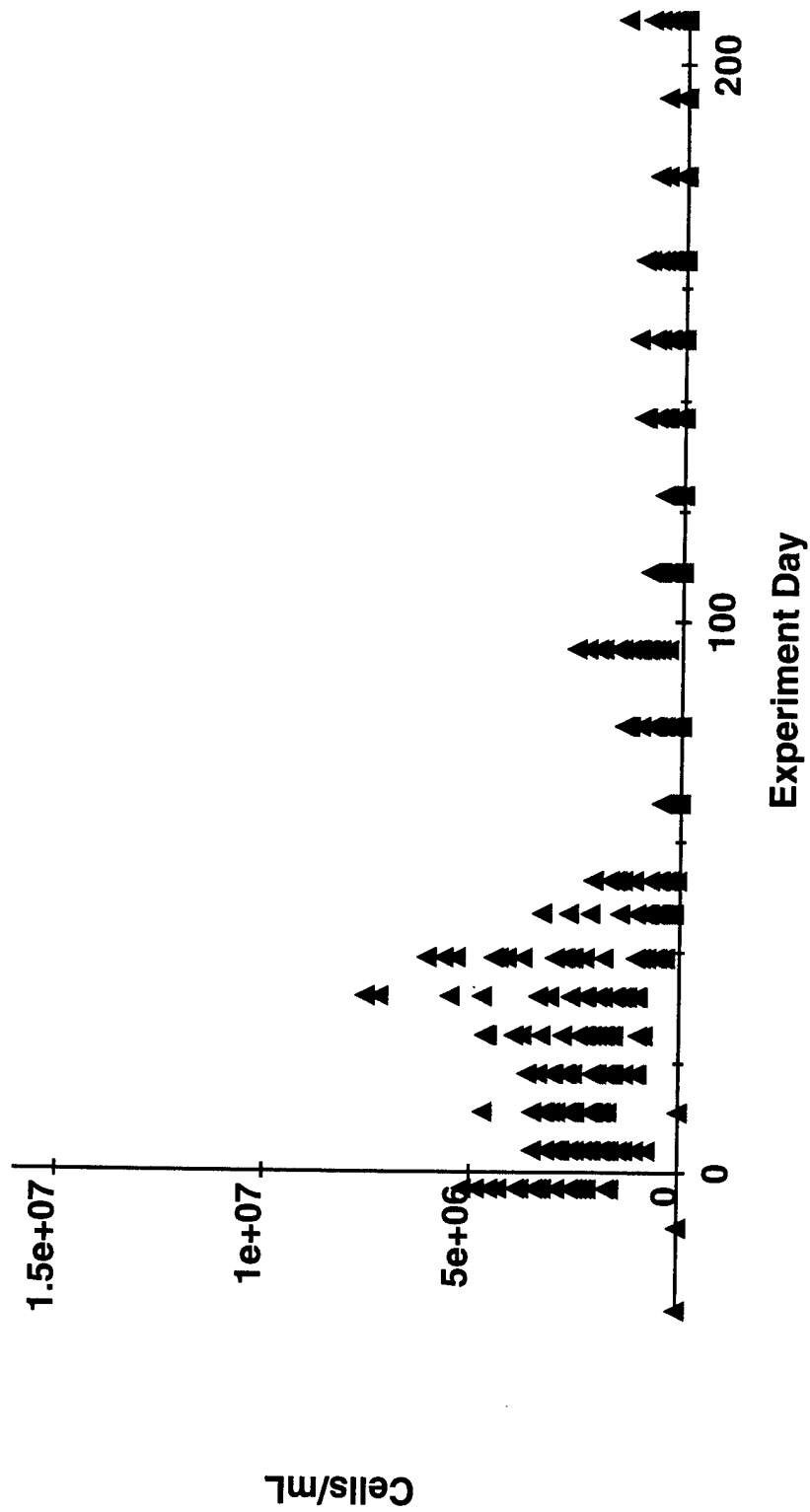
Figure 11b. Periphyton - Diatoms
Pennate - Mid-Dose
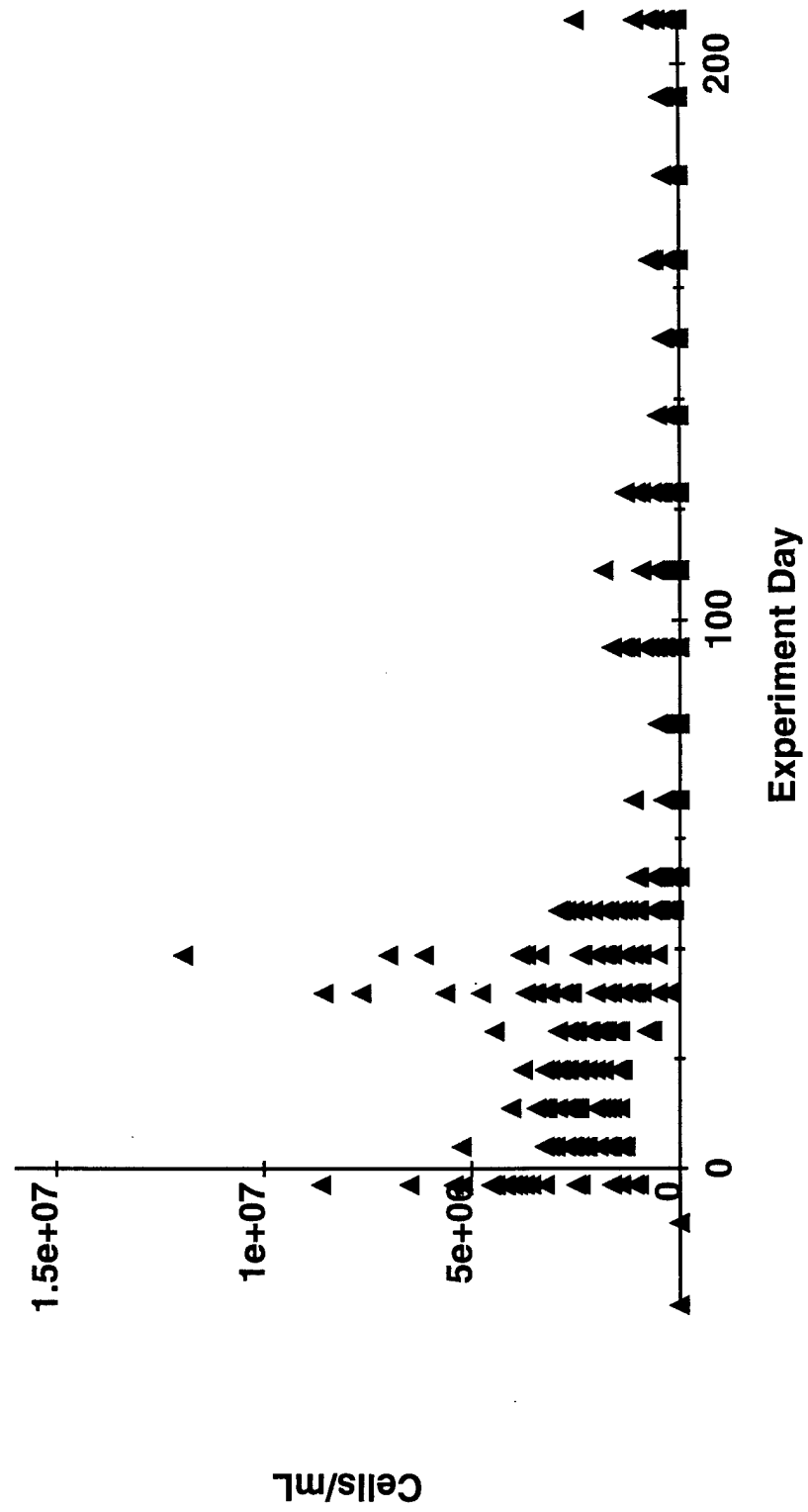
Figure 11c. Periphyton - Diatoms
Pennate - Low Dose
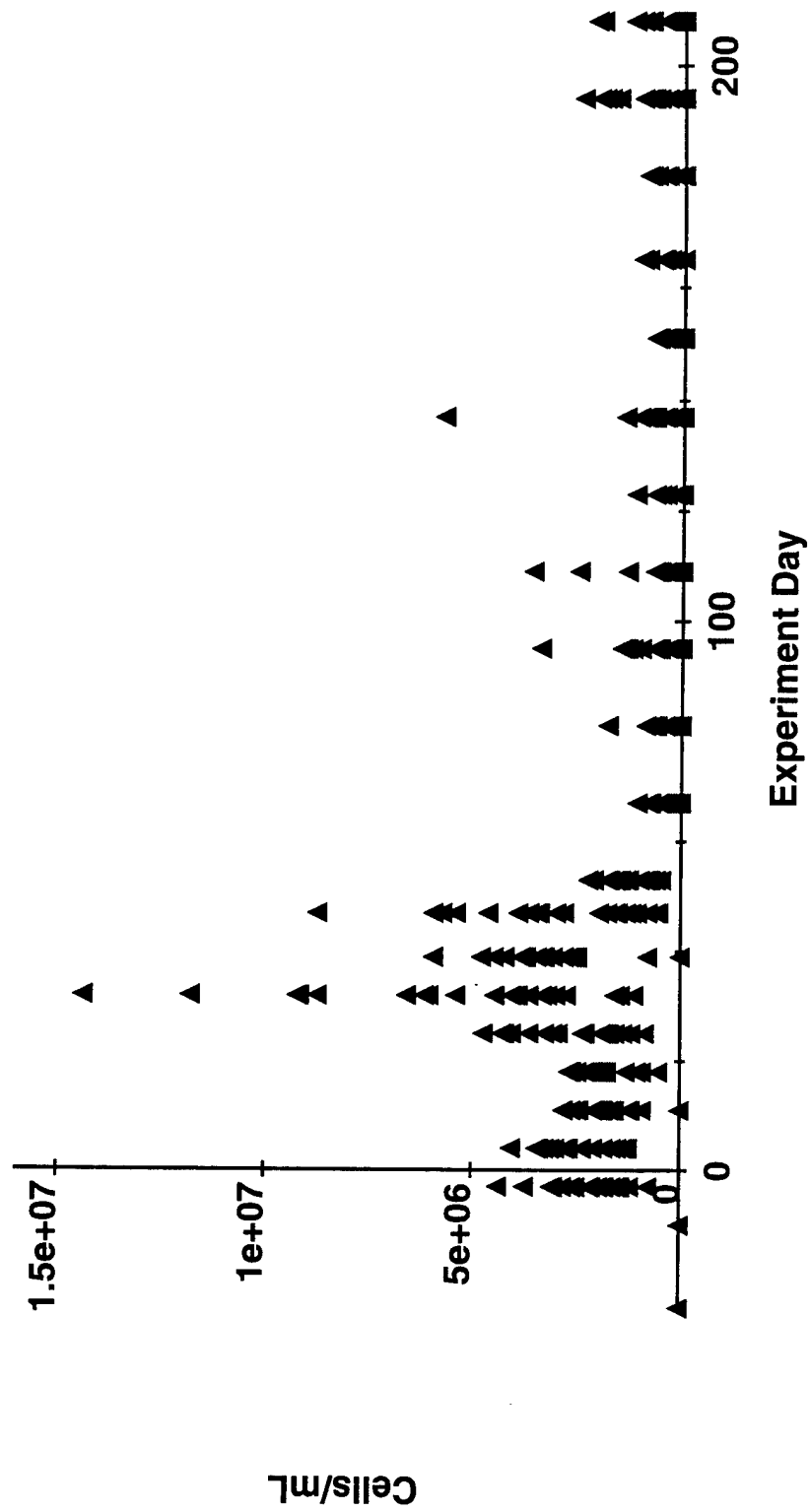
Figure 11d. Periphyton - Diatoms
Pennate - Undosed
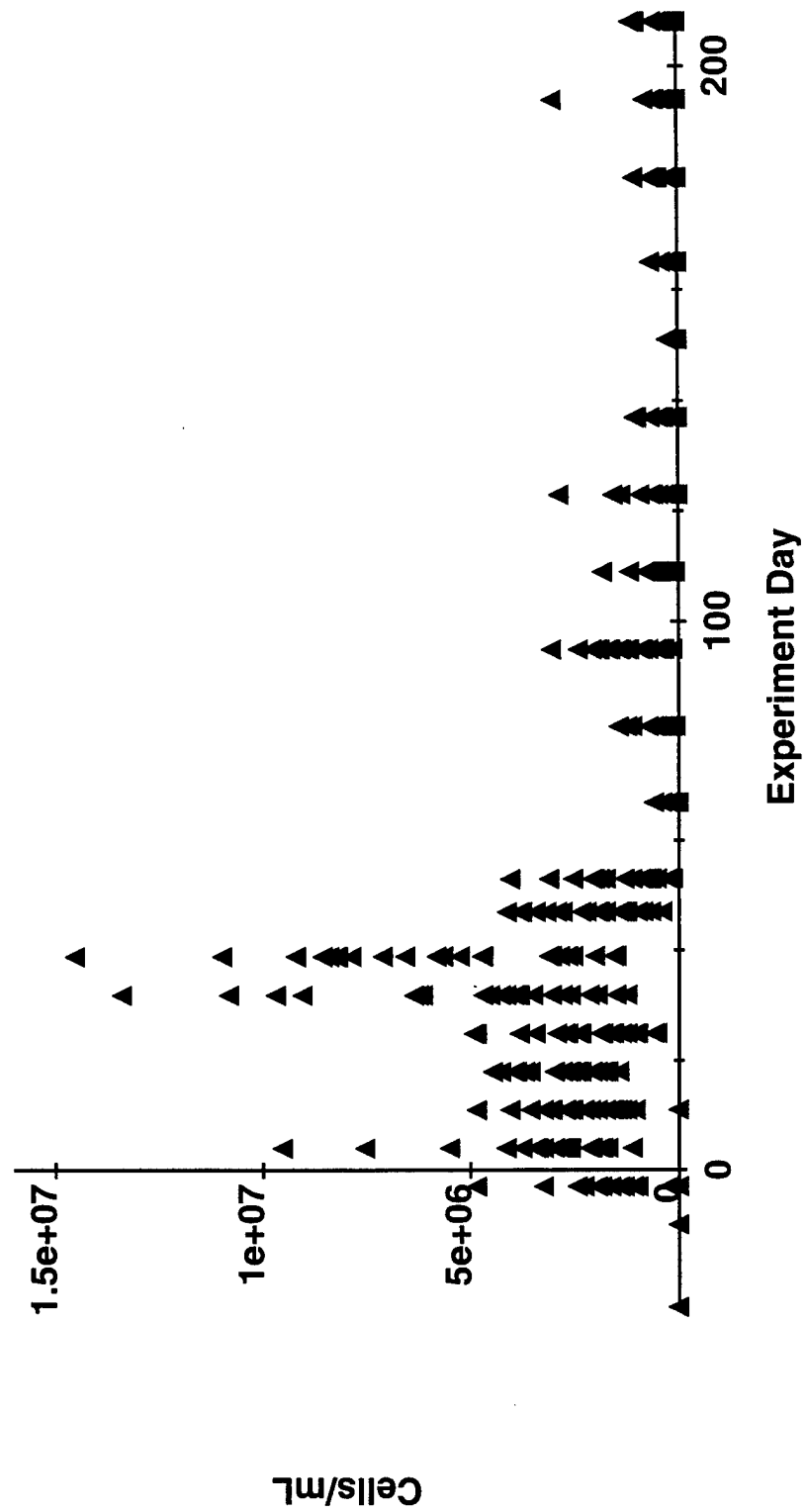
Figure 12a. Periphyton - Flagellates
Autotrophs, High Dose
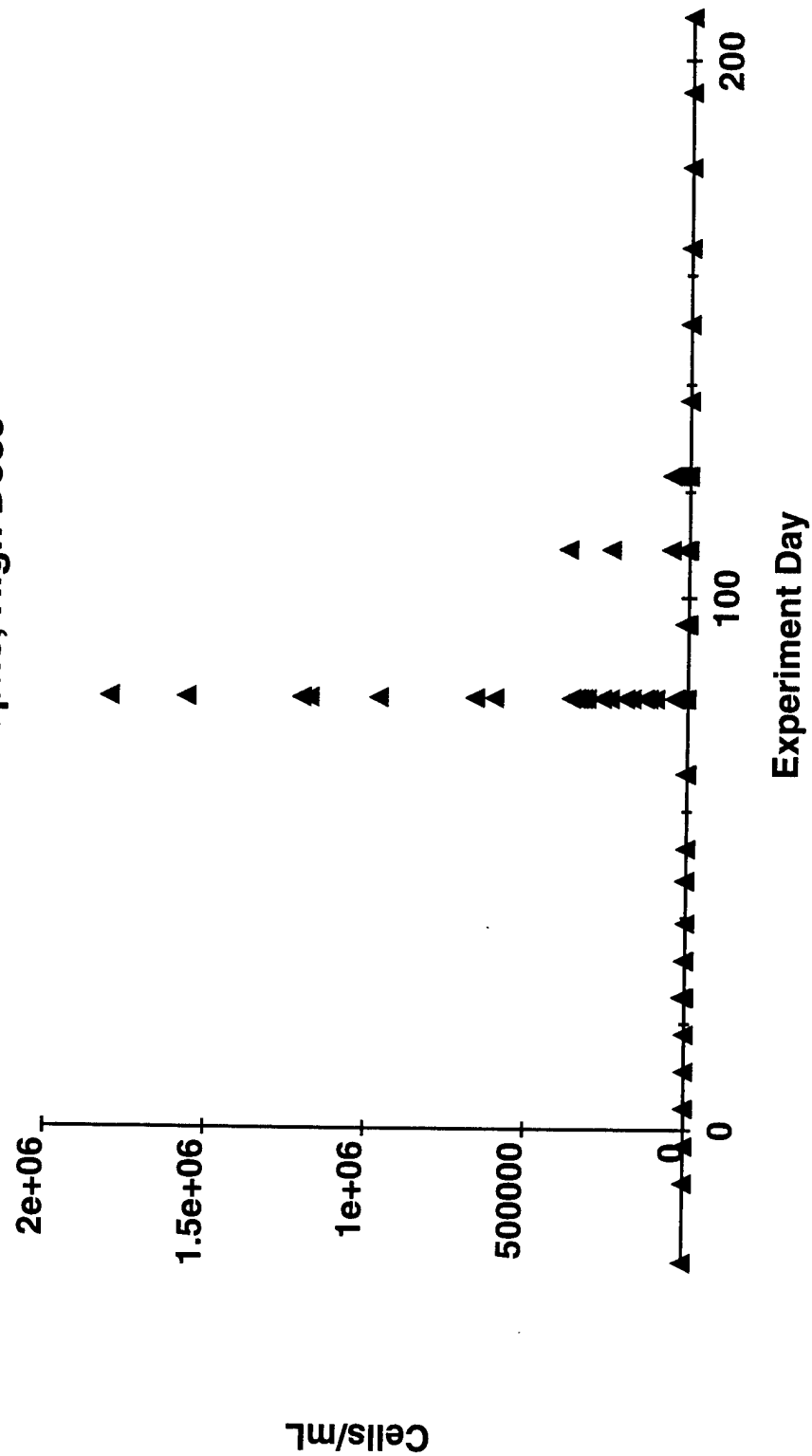
Figure 12b. Periphyton - Flagellates
Autotrophs - Mid-Dose
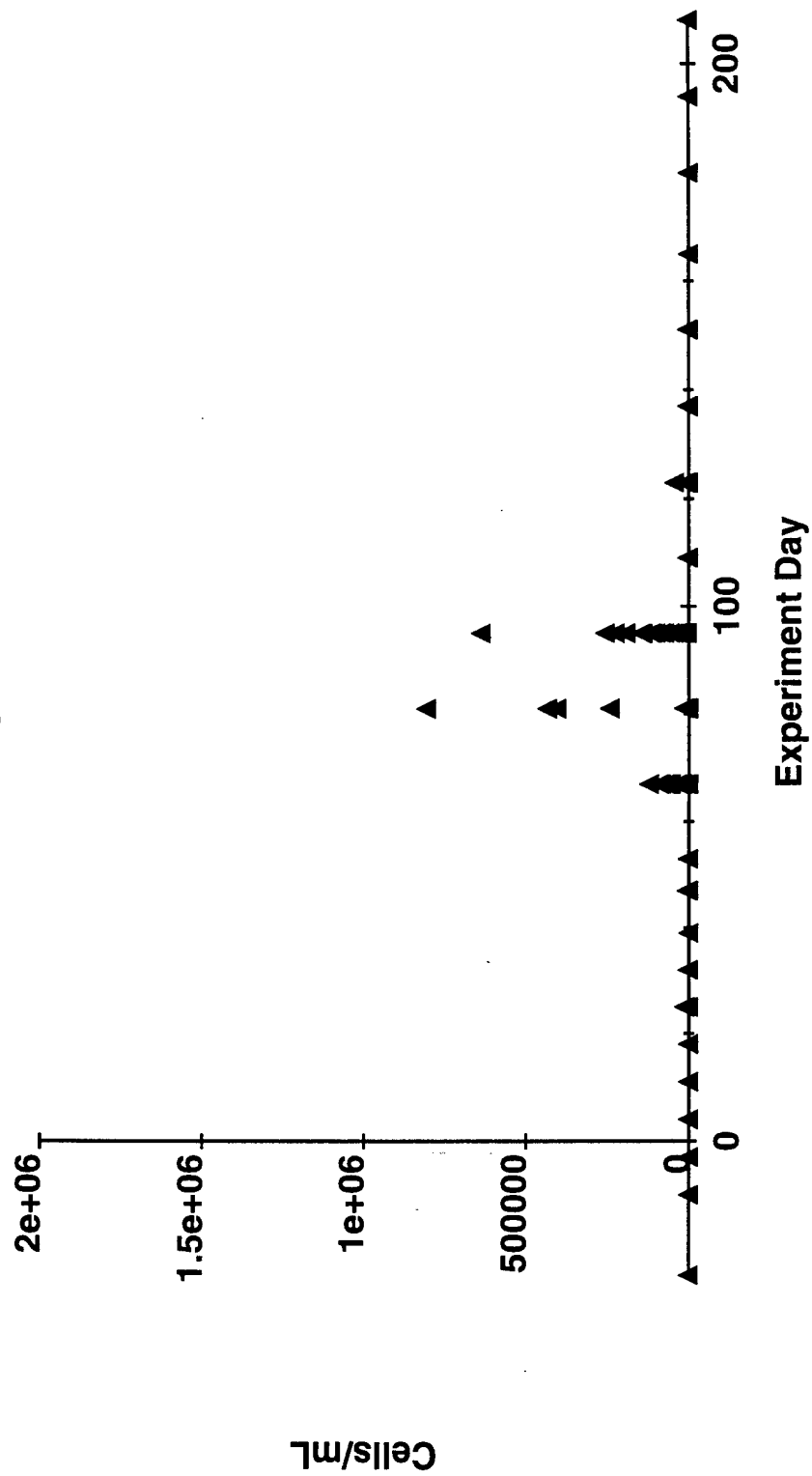
Figure 12c. Periphyton - Flagellates
Autotrophs - Low Dose

Cells/mL

Experiment Day
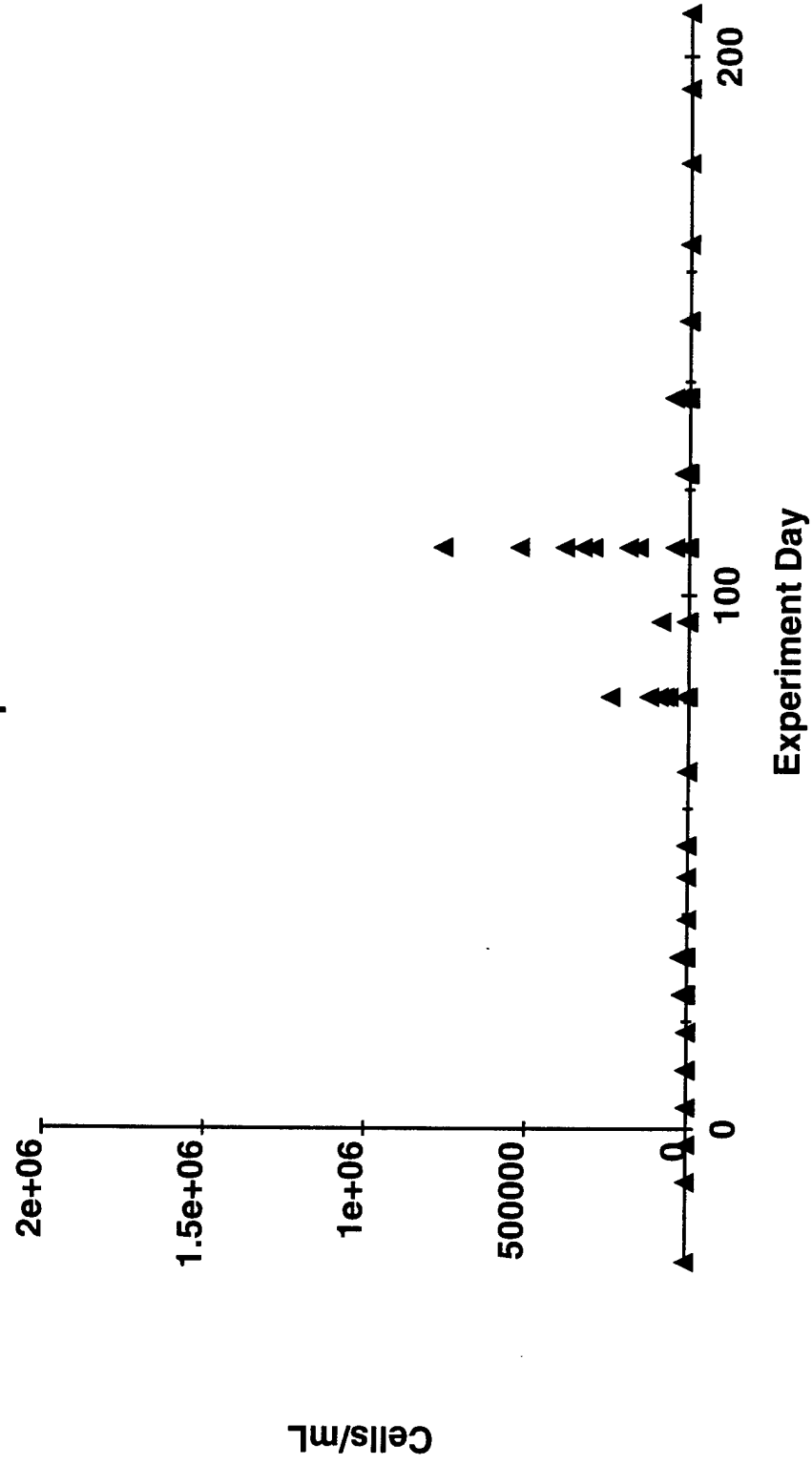
Figure 12d. Periphyton - Flagellates
Autotrophs - Undosed

Cells/mL

Experiment Day
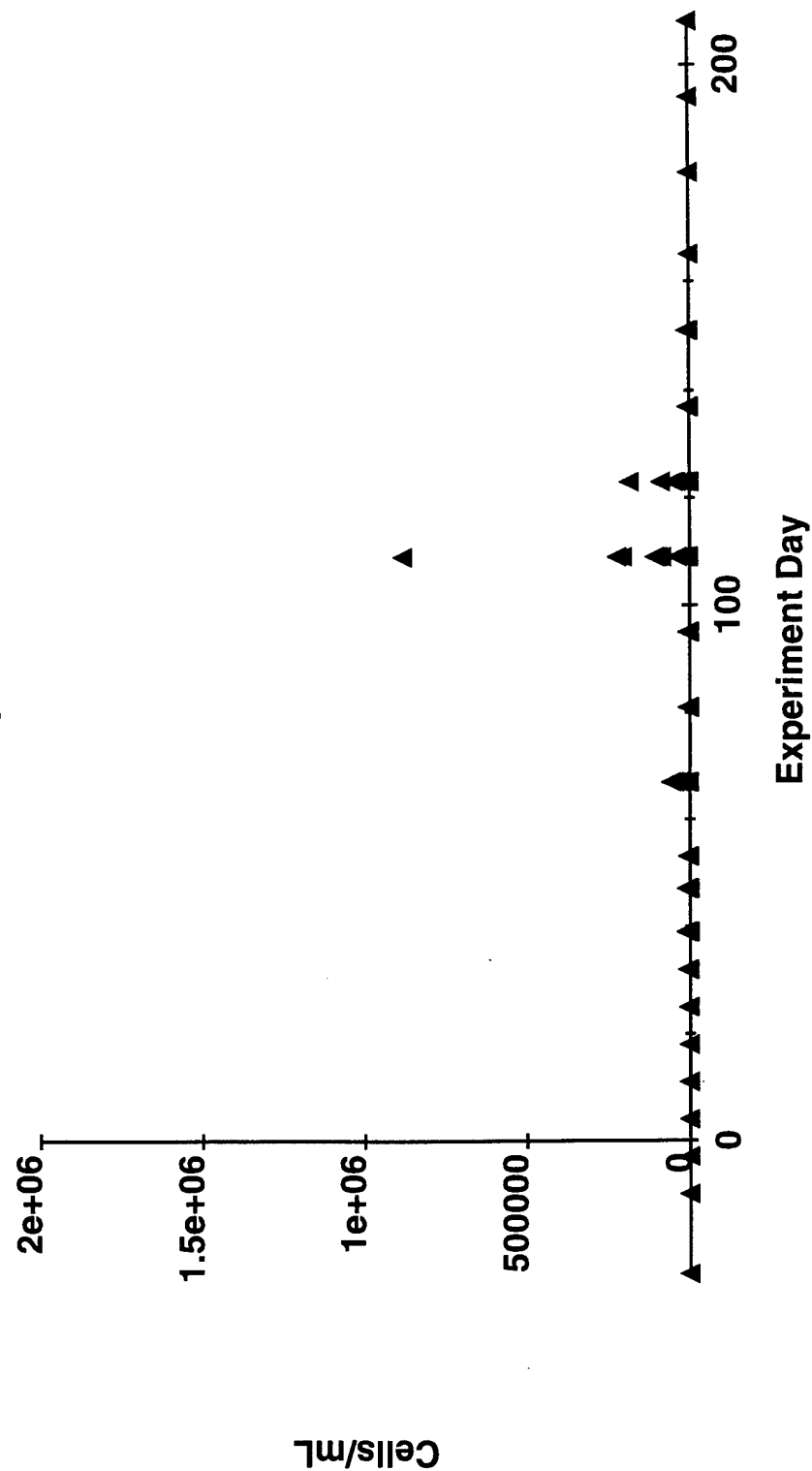
Figure 13a. Periphyton - Flagellates
Euglenoids, High Dose
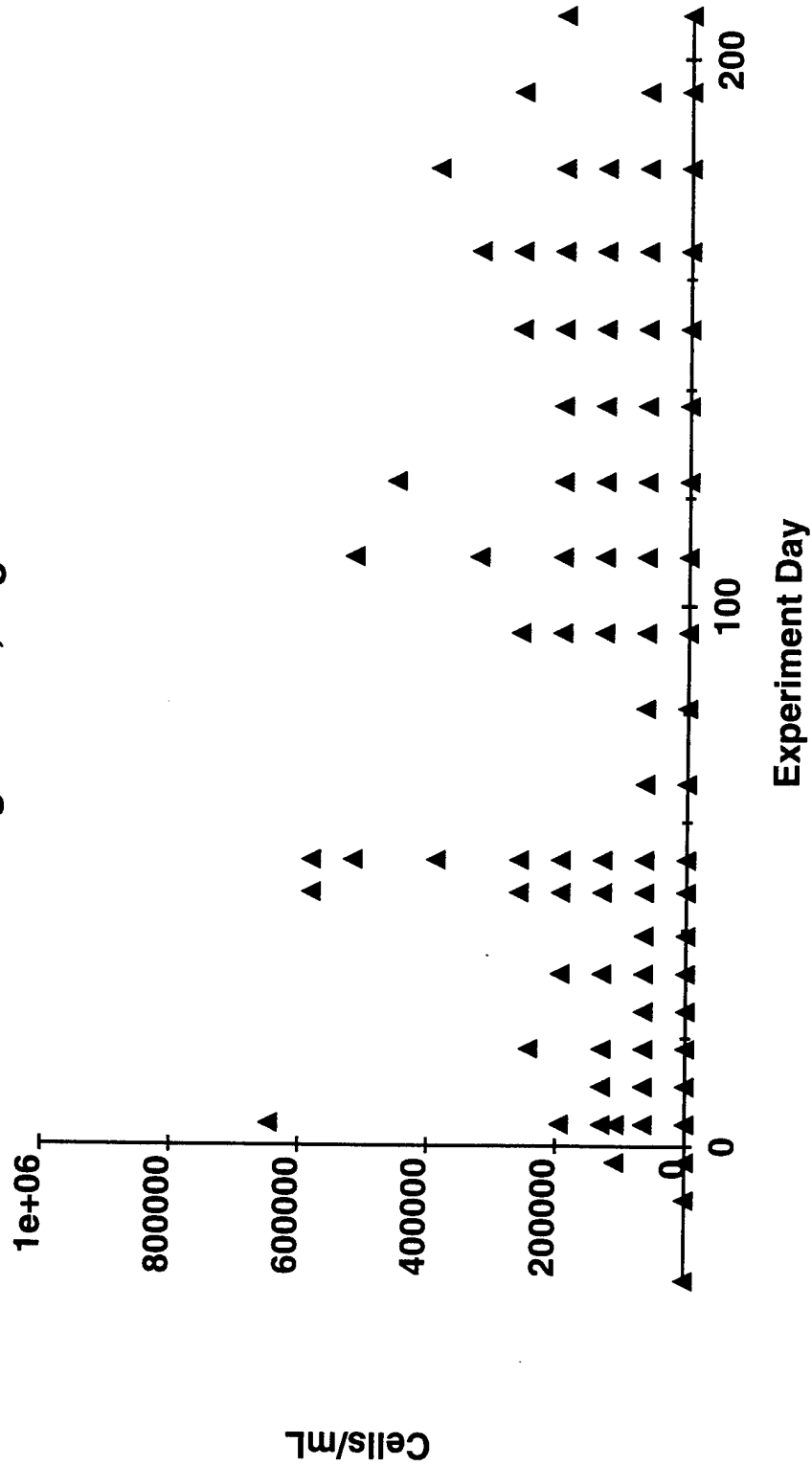
Figure 13b. Periphyton - Flagellates
Euglenoids - Mid-Dose
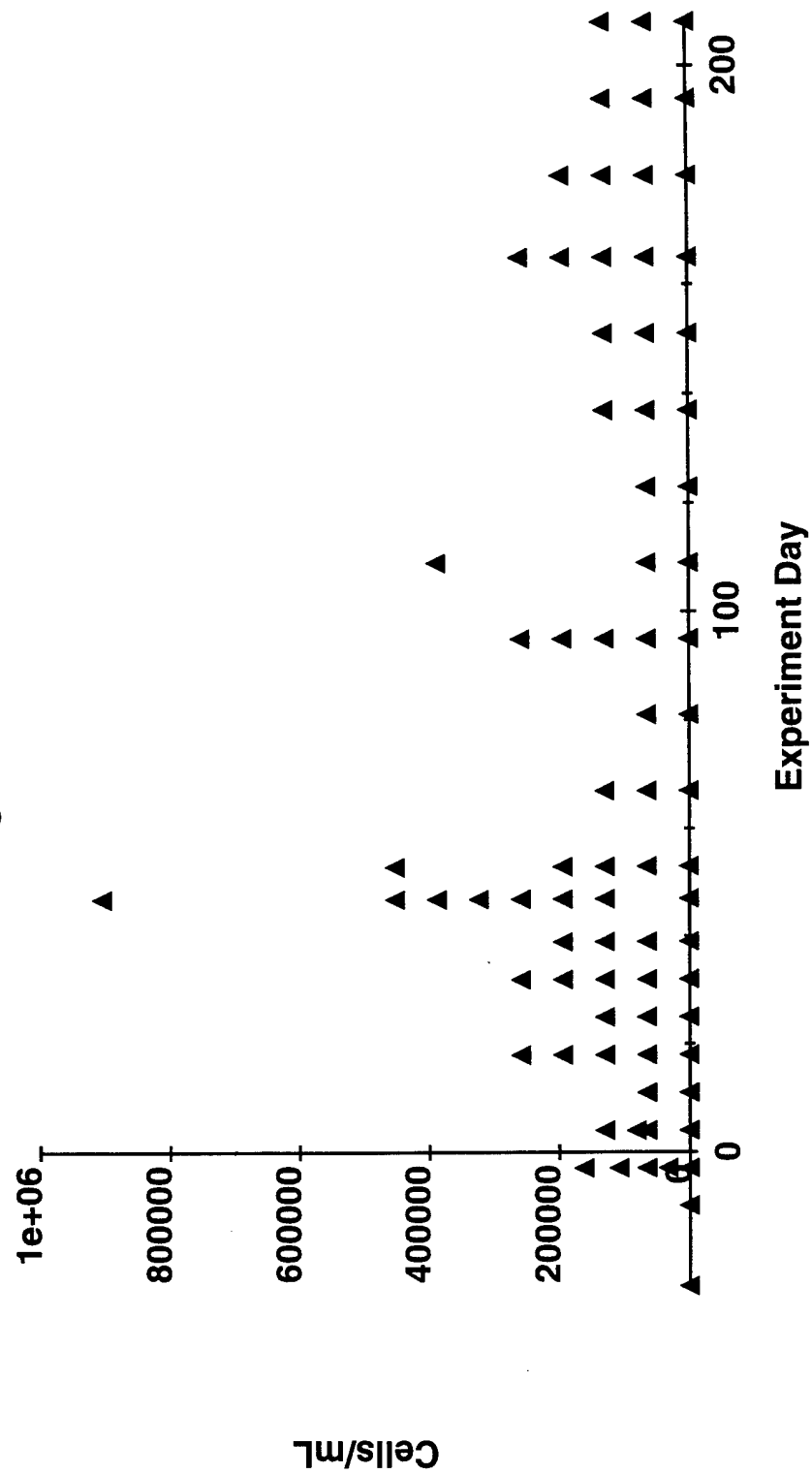
Figure 13c. Periphyton - Flagellates
Euglenoids - Low Dose
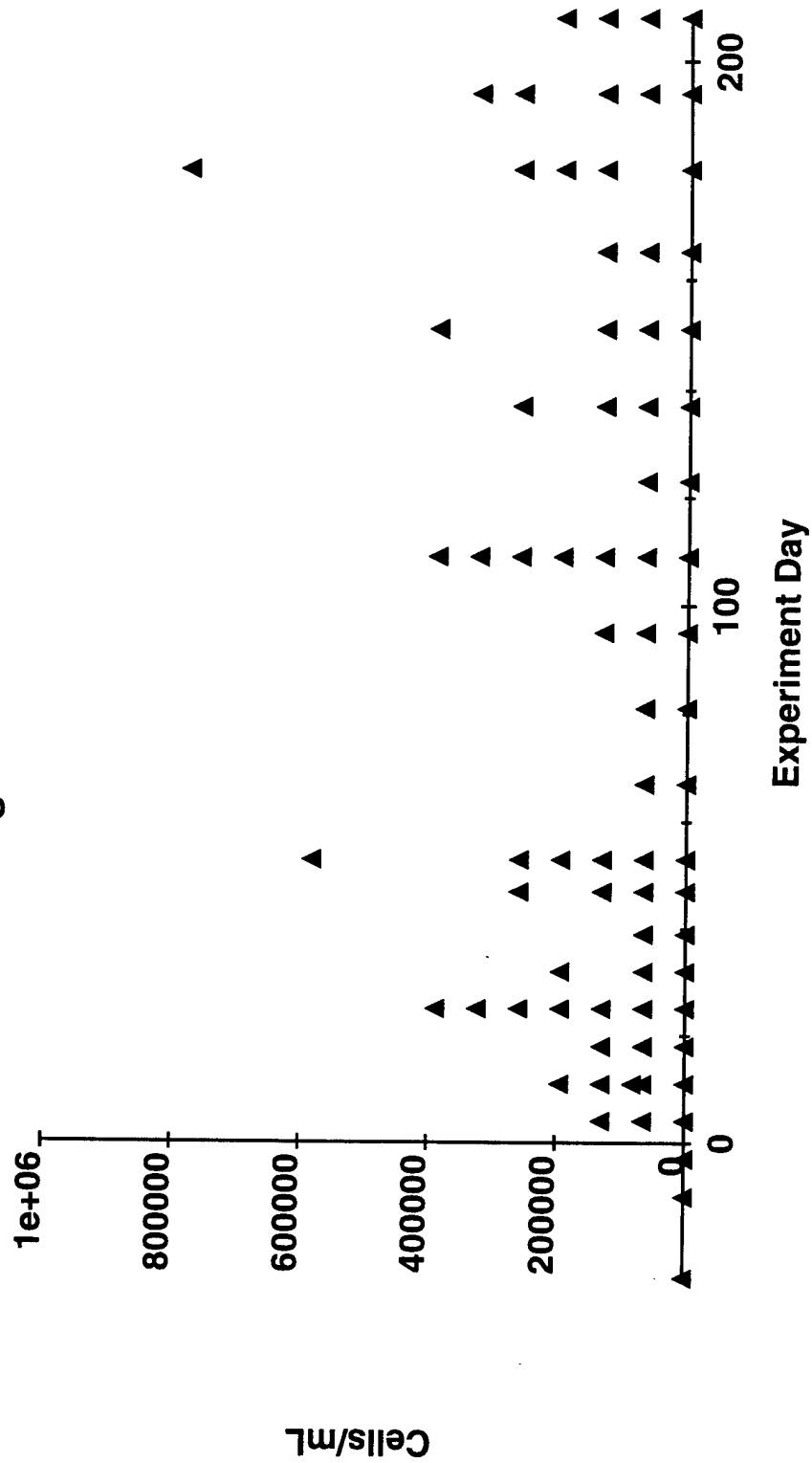
Figure 13d. Periphyton - Flagellates
Euglenoids - Undosed
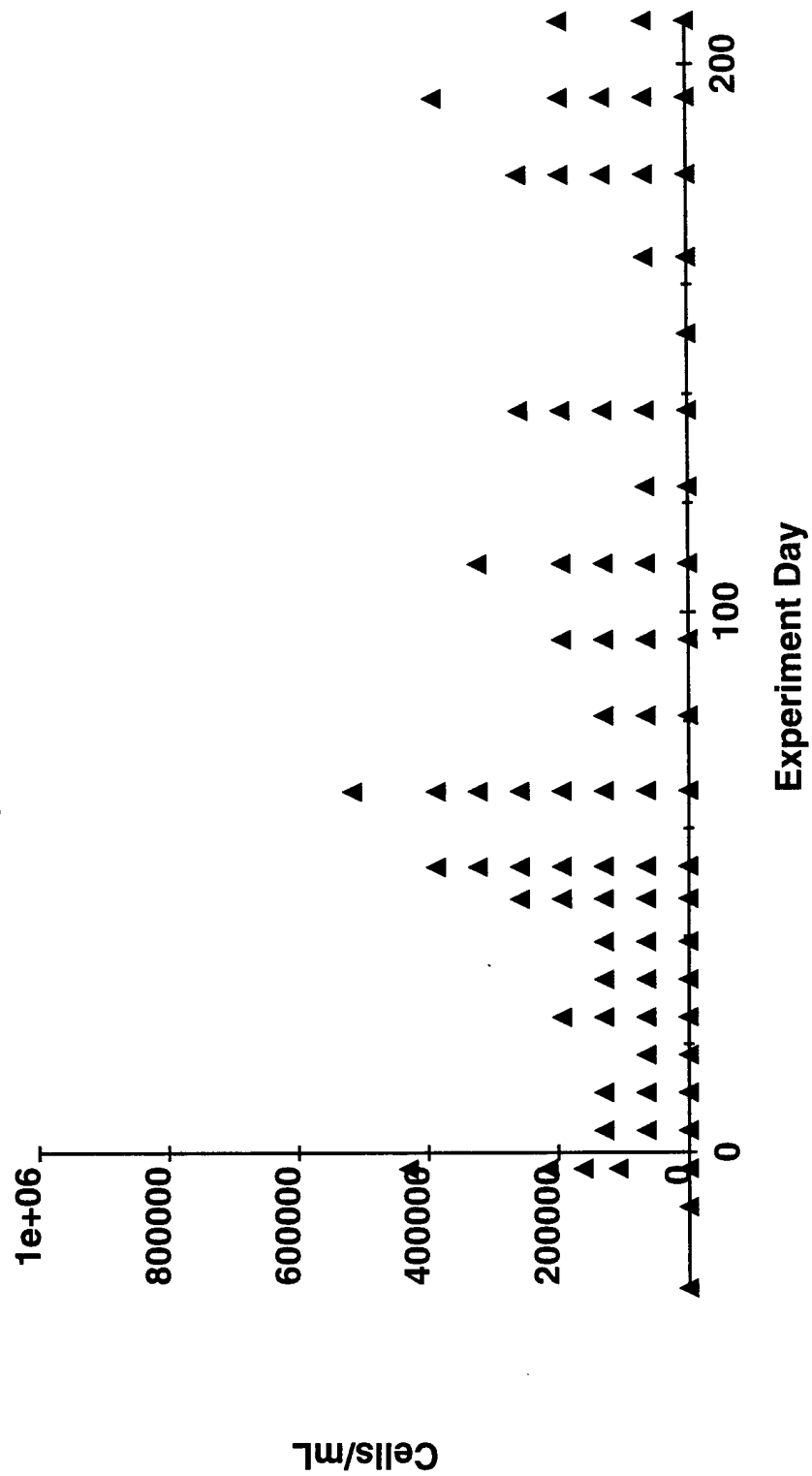
Figure 14a. Periphyton - Flagellates
Heterotrophs, High Dose
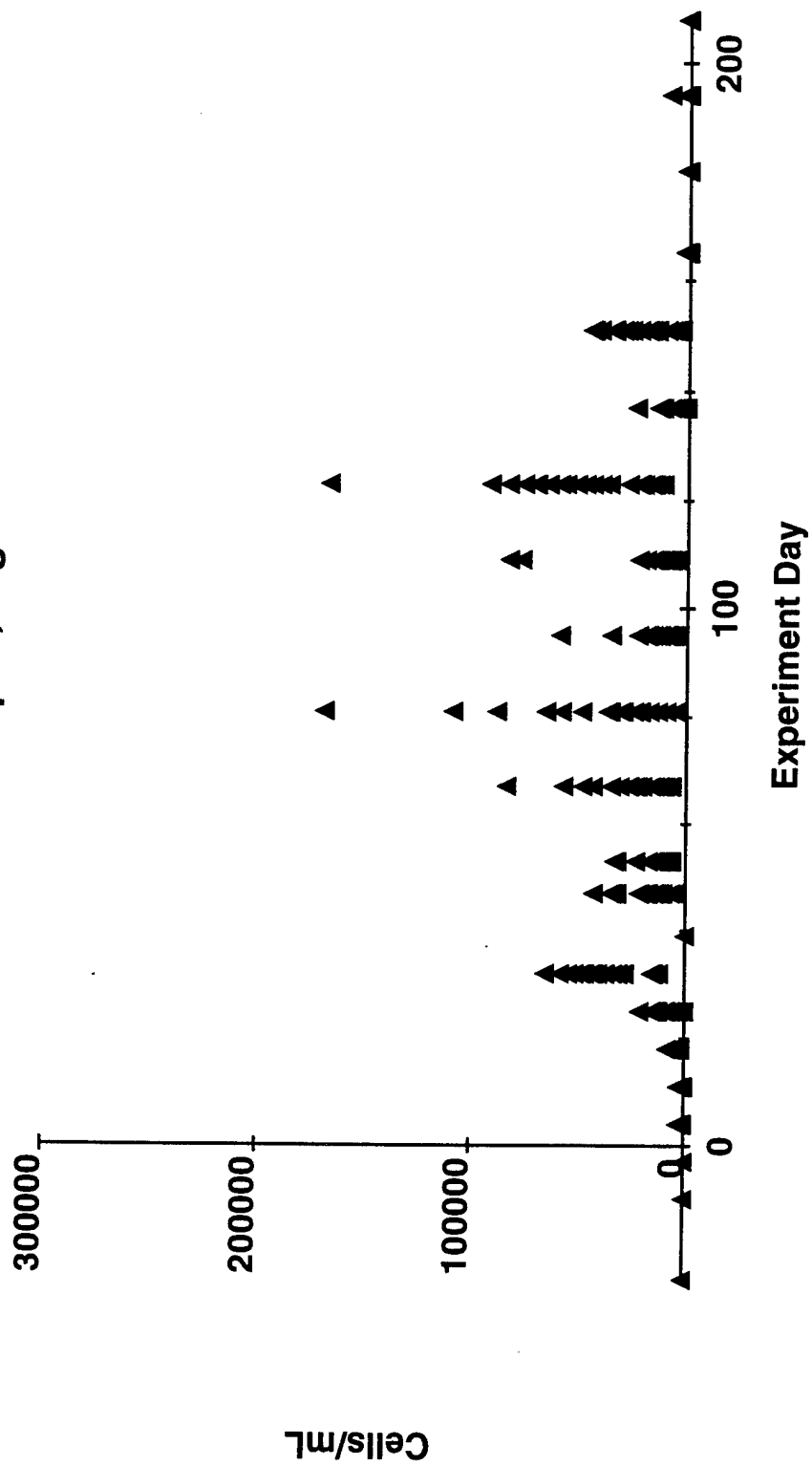
Figure 14b. Periphyton - Flagellates
Heterotrophs - Mid-Dose
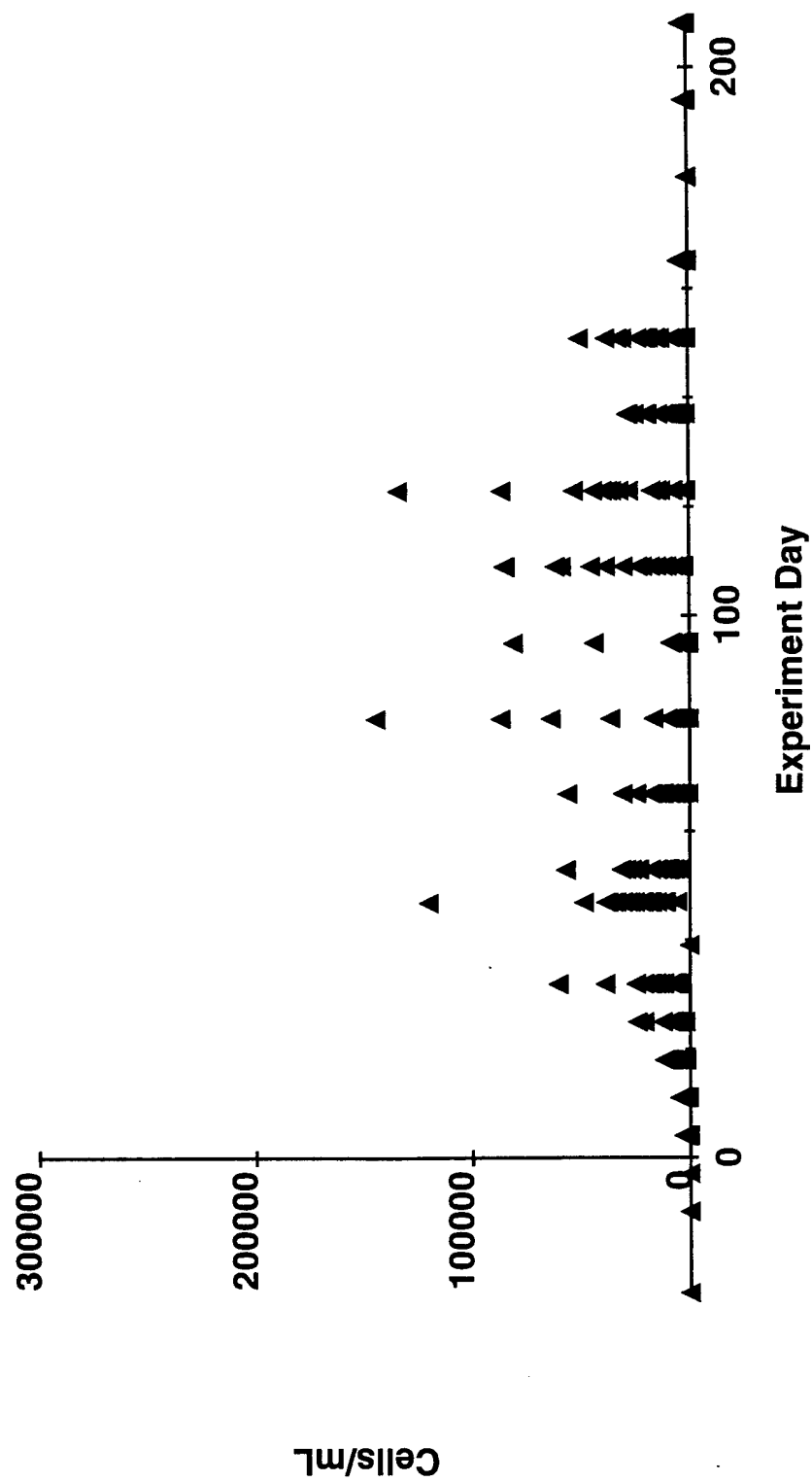
Figure 14c. Periphyton - Flagellates
Heterotrophs - Low Dose
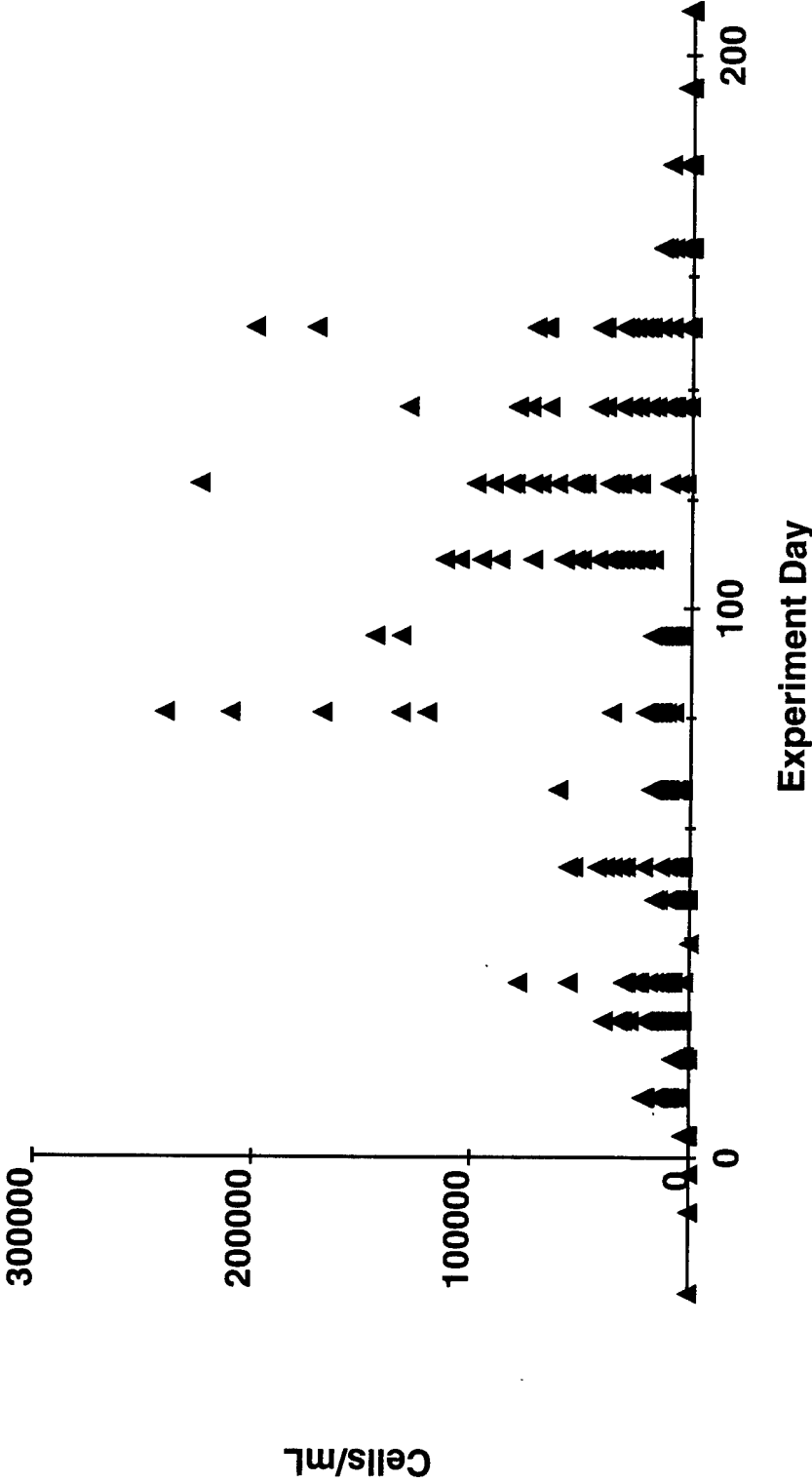
Figure 14d. Periphyton - Flagellates
Heterotrophs - Undosed
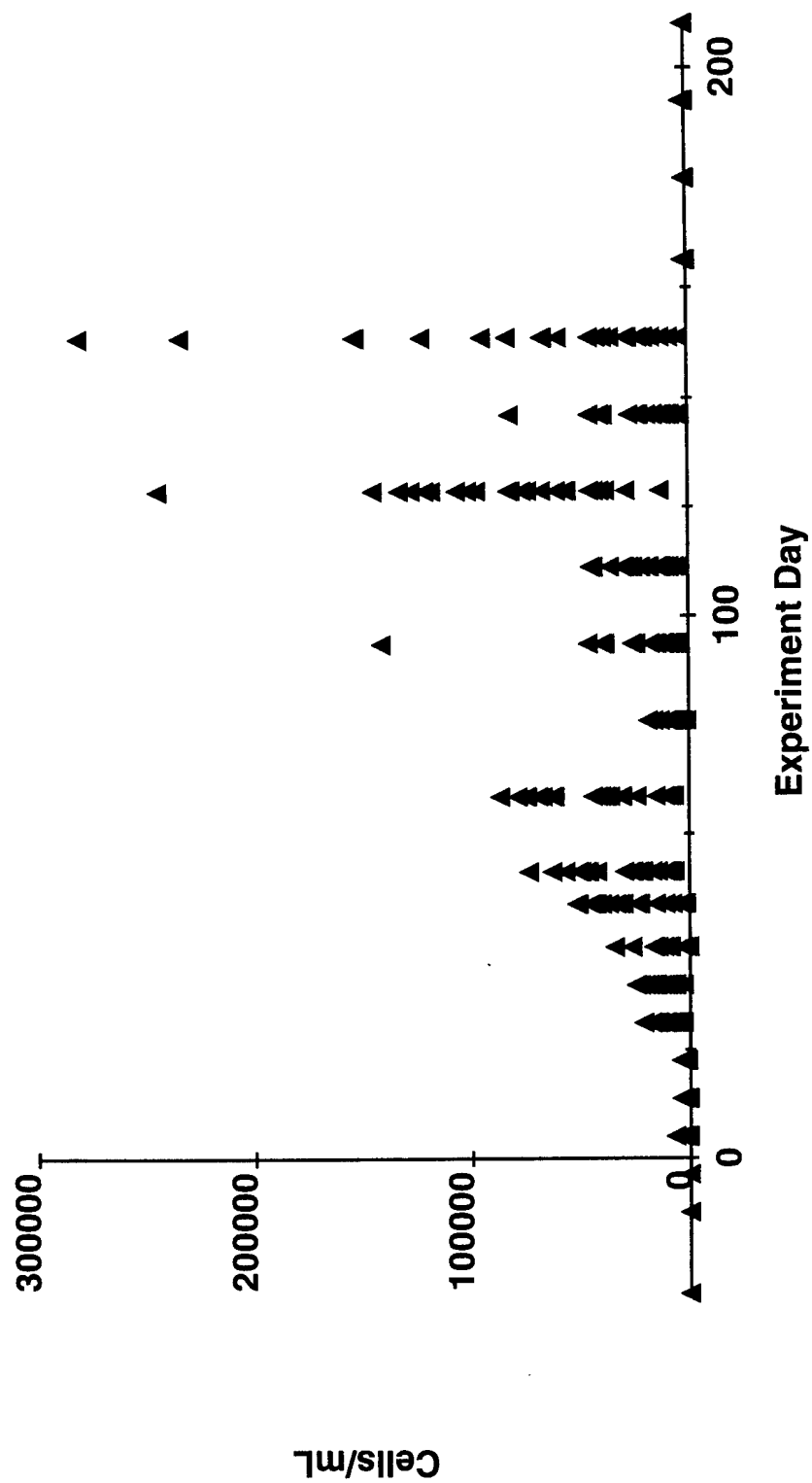
Figure 15a. Periphyton - Green Algae
Ankistrodesmus, High Dose
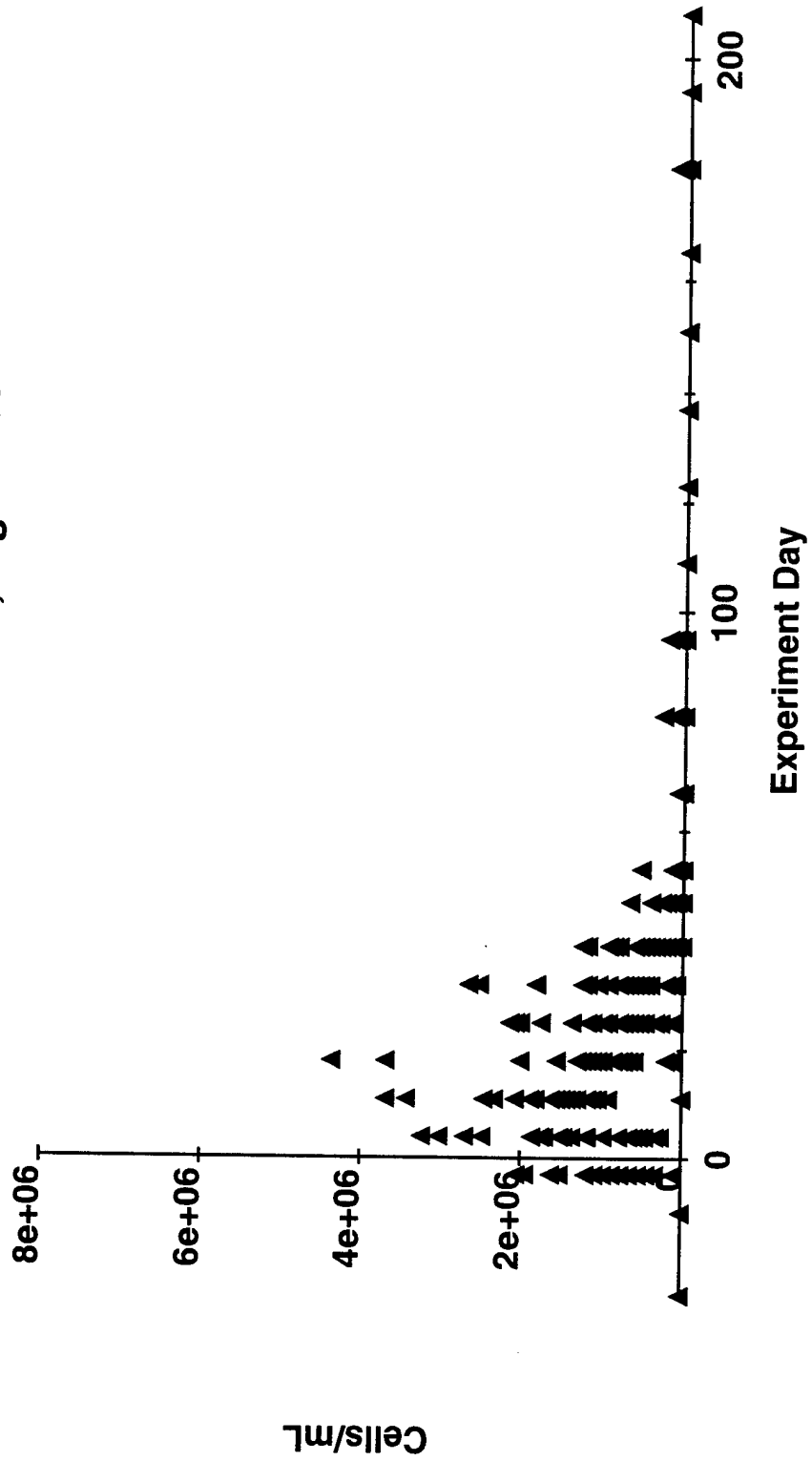
Figure 15b. Periphyton - Green Algae
Ankistrodesmus - Mid-Dose
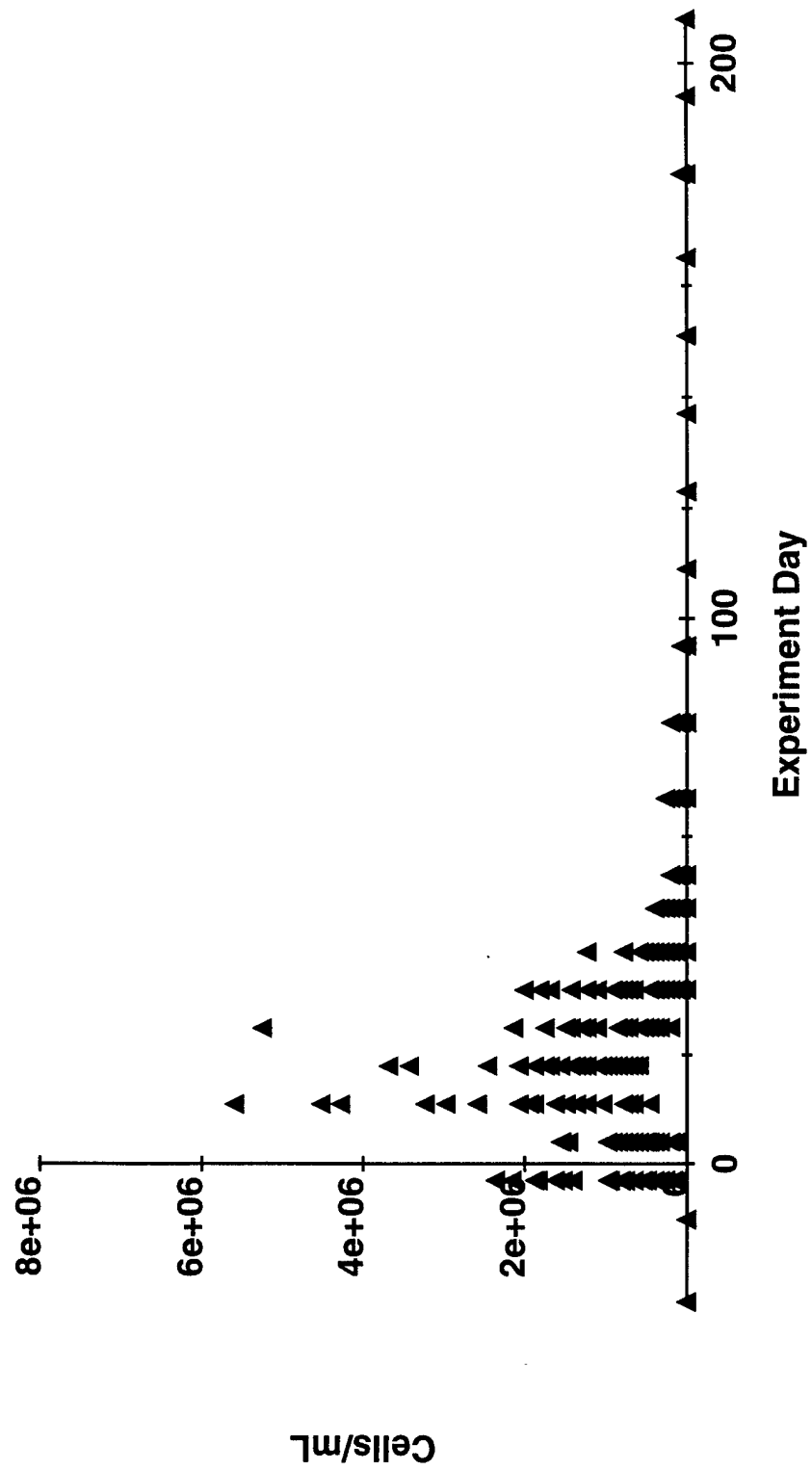
Figure 15c. Periphyton - Green Algae
Ankistrodesmus - Low Dose
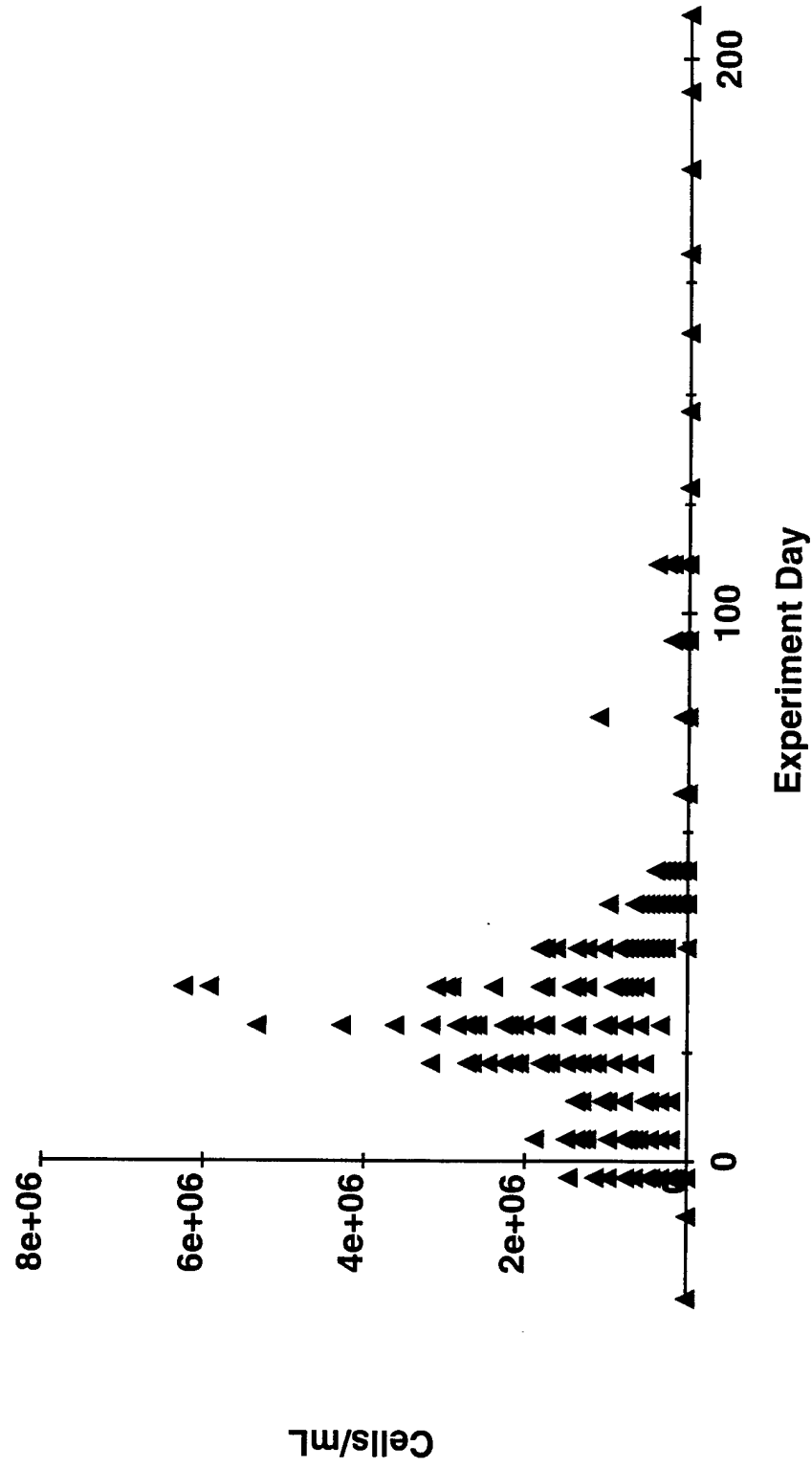
Figure 15d. Periphyton - Green Algae
Ankistrodesmus - Undosed
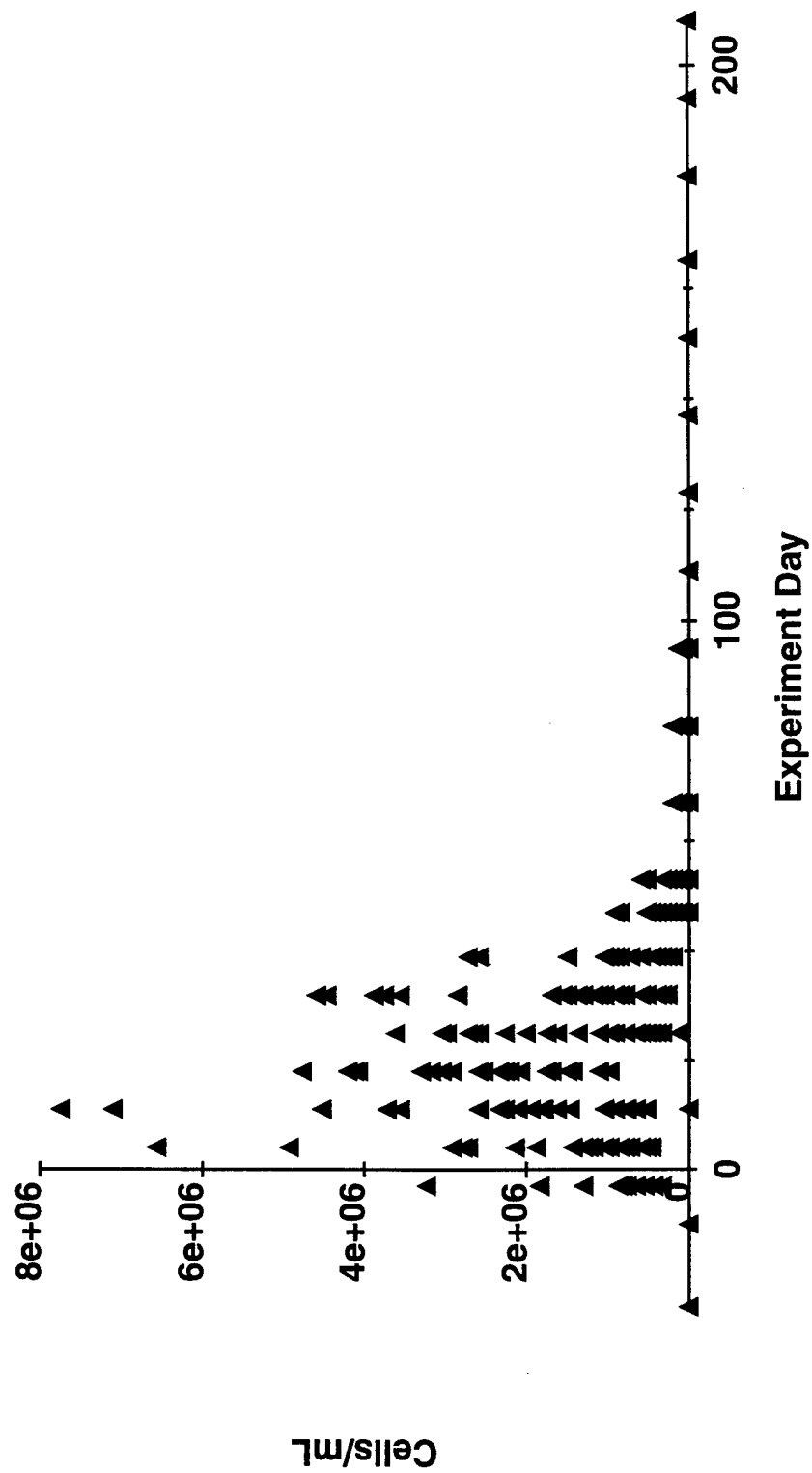
Figure 16a. Periphyton - Green Algae
Chlamydomonas, High Dose
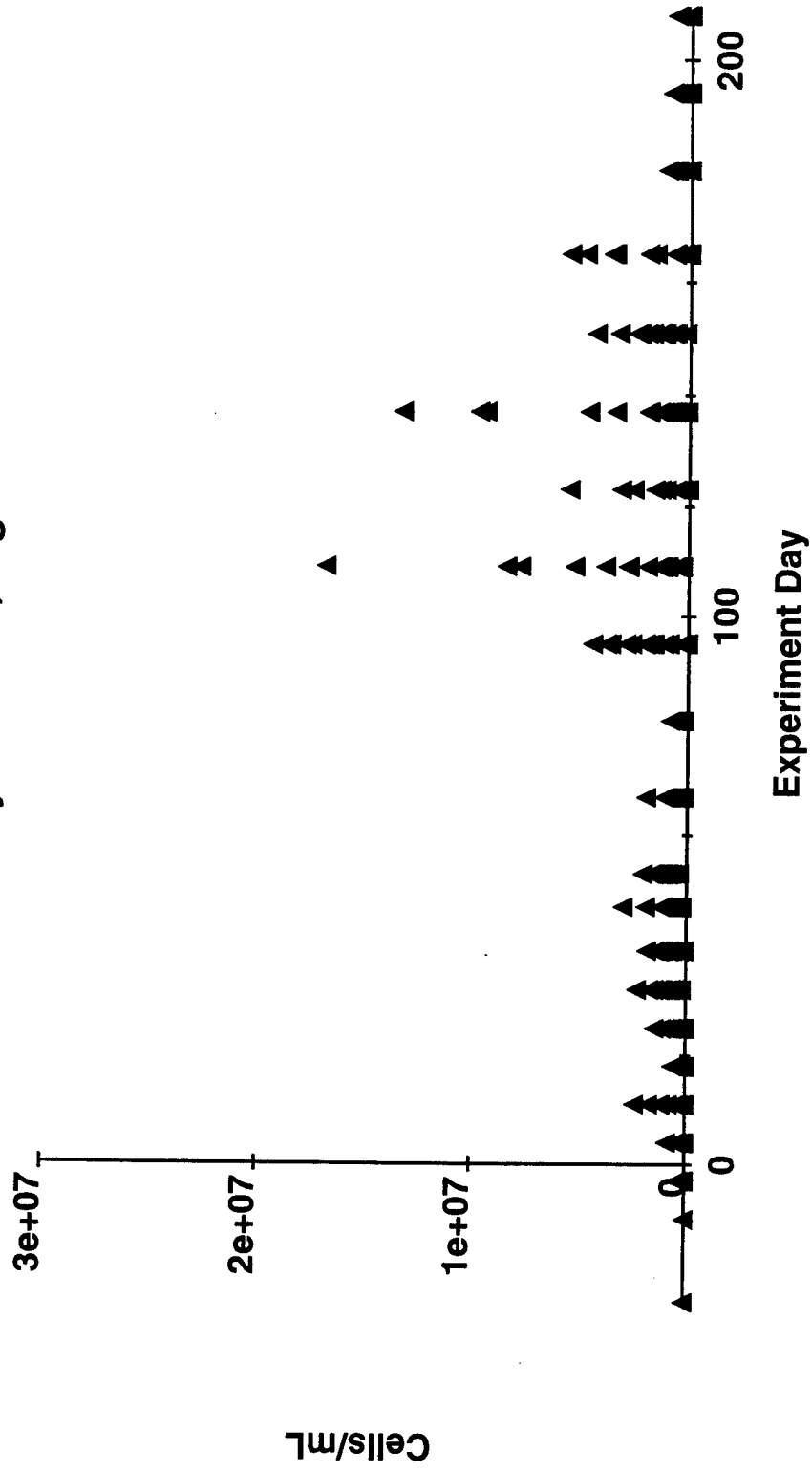
Figure 16b. Periphyton - Green Algae
Chlamydomonas - Mid-Dose
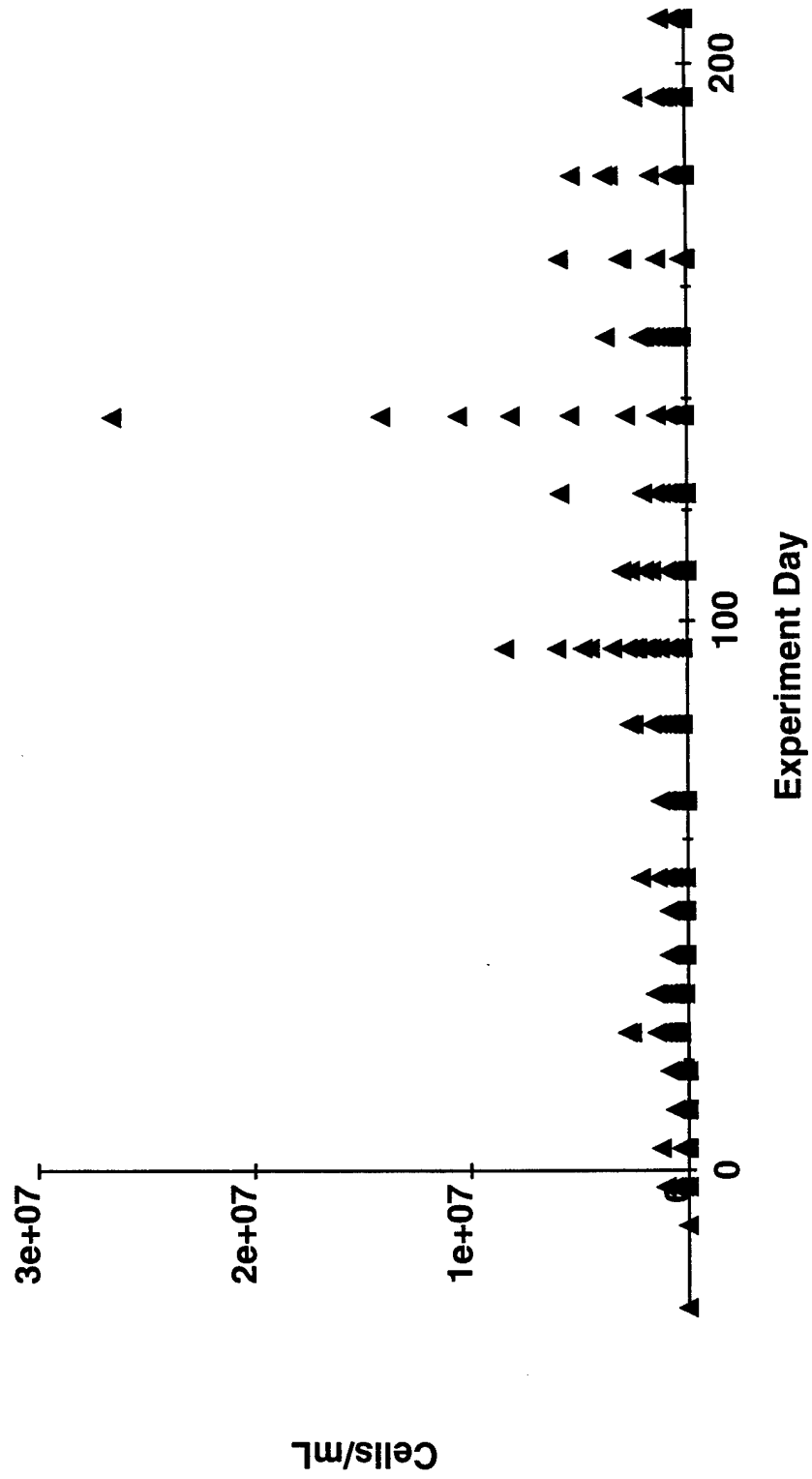
Figure 16c. Periphyton - Green Algae
Chlamydomonas - Low Dose
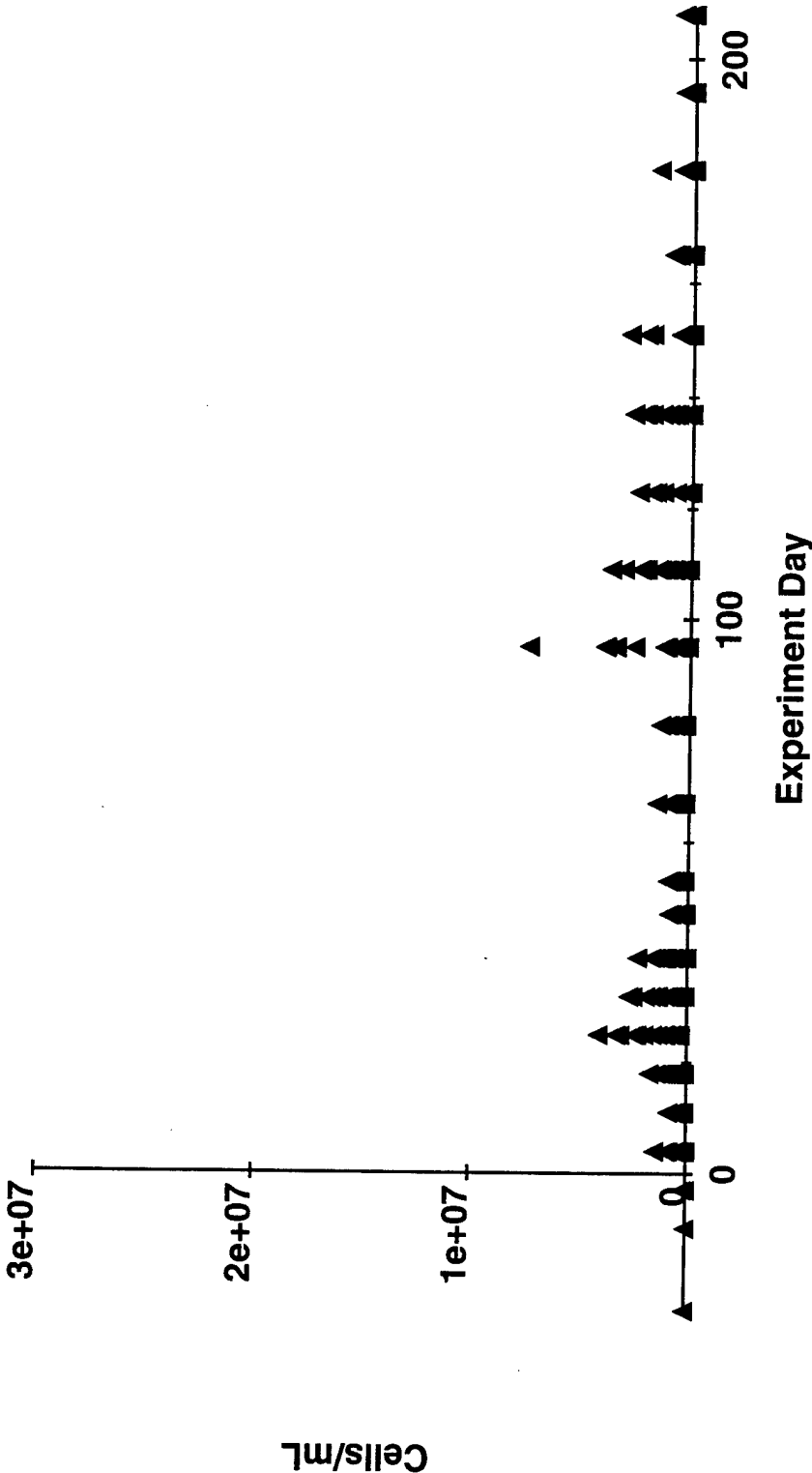
Figure 16d. Periphyton - Green Algae
Chlamydomonas - Undosed
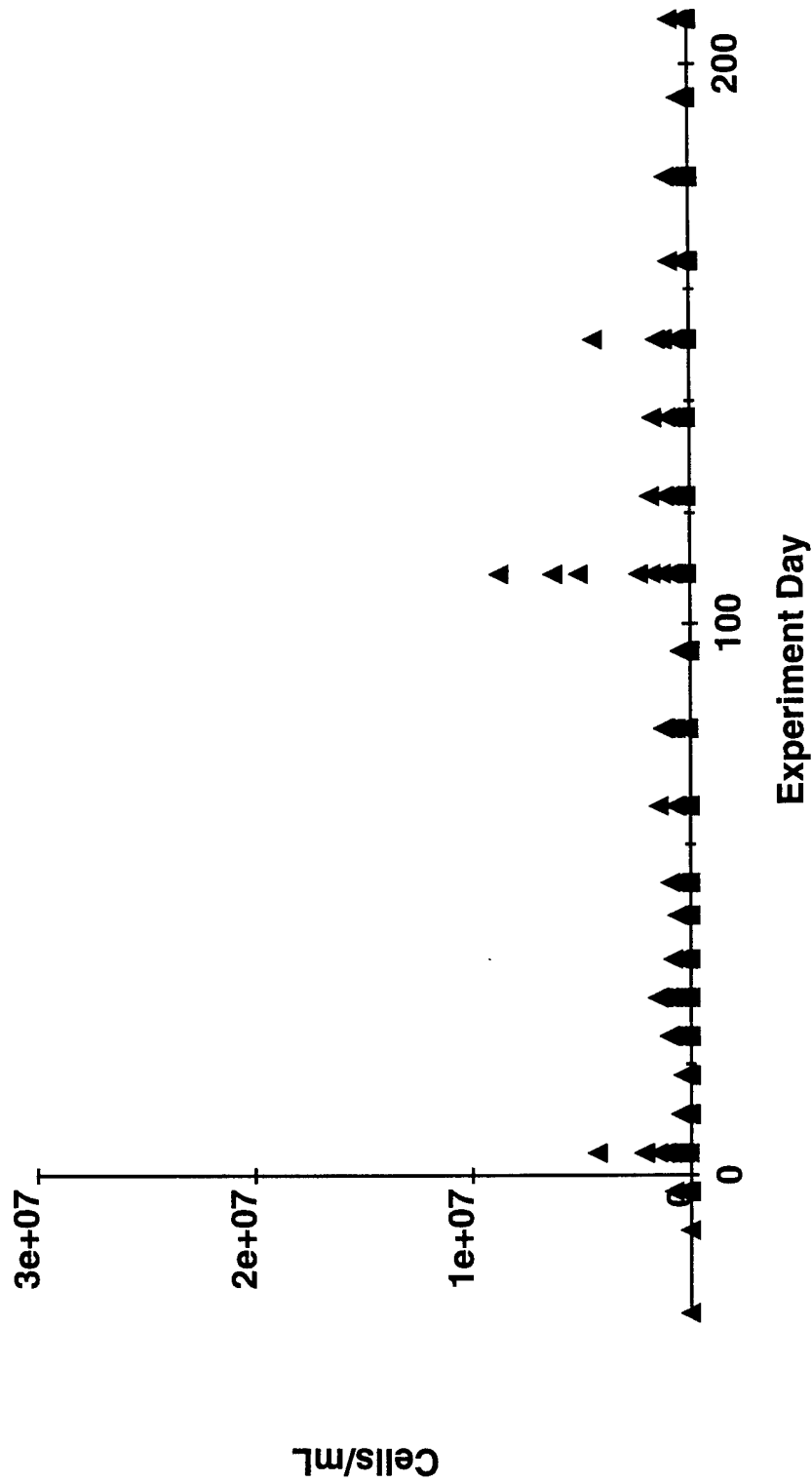
Figure 17a. Periphyton - Green Algae
Filamentous, High Dose
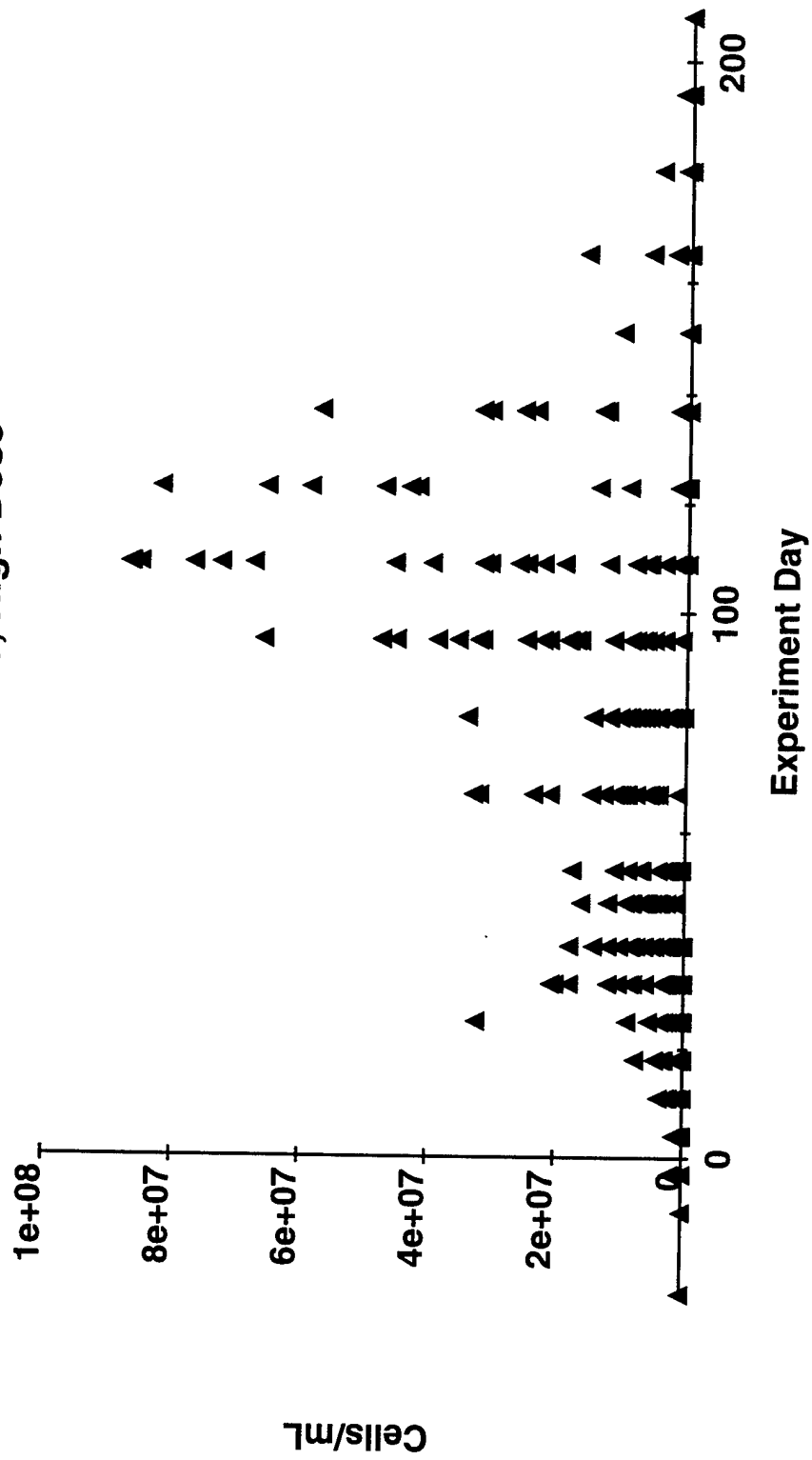
Figure 17b. Periphyton - Green Algae
Filamentous - Mid-Dose
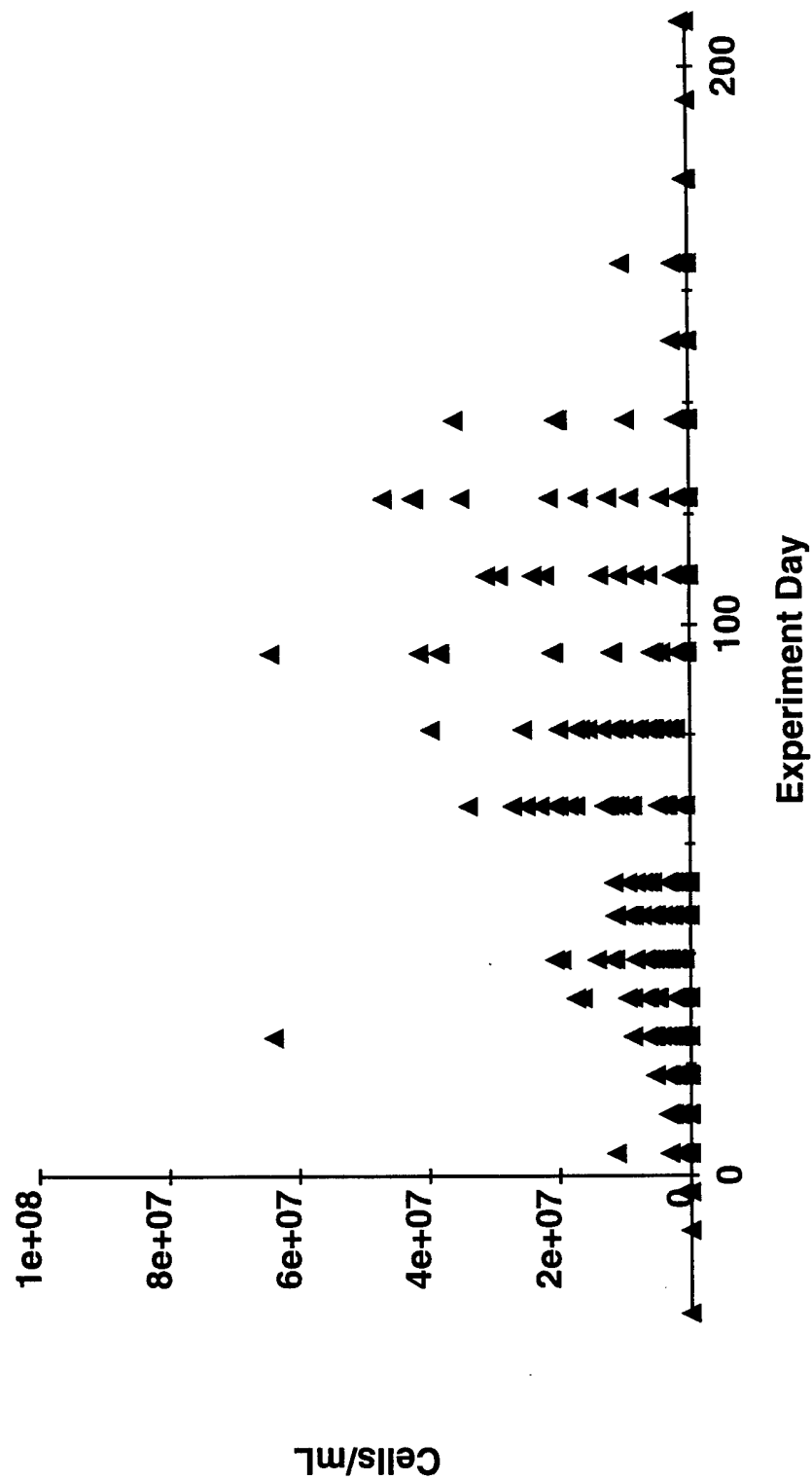
Figure 17c. Periphyton - Green Algae
Filamentous - Low Dose
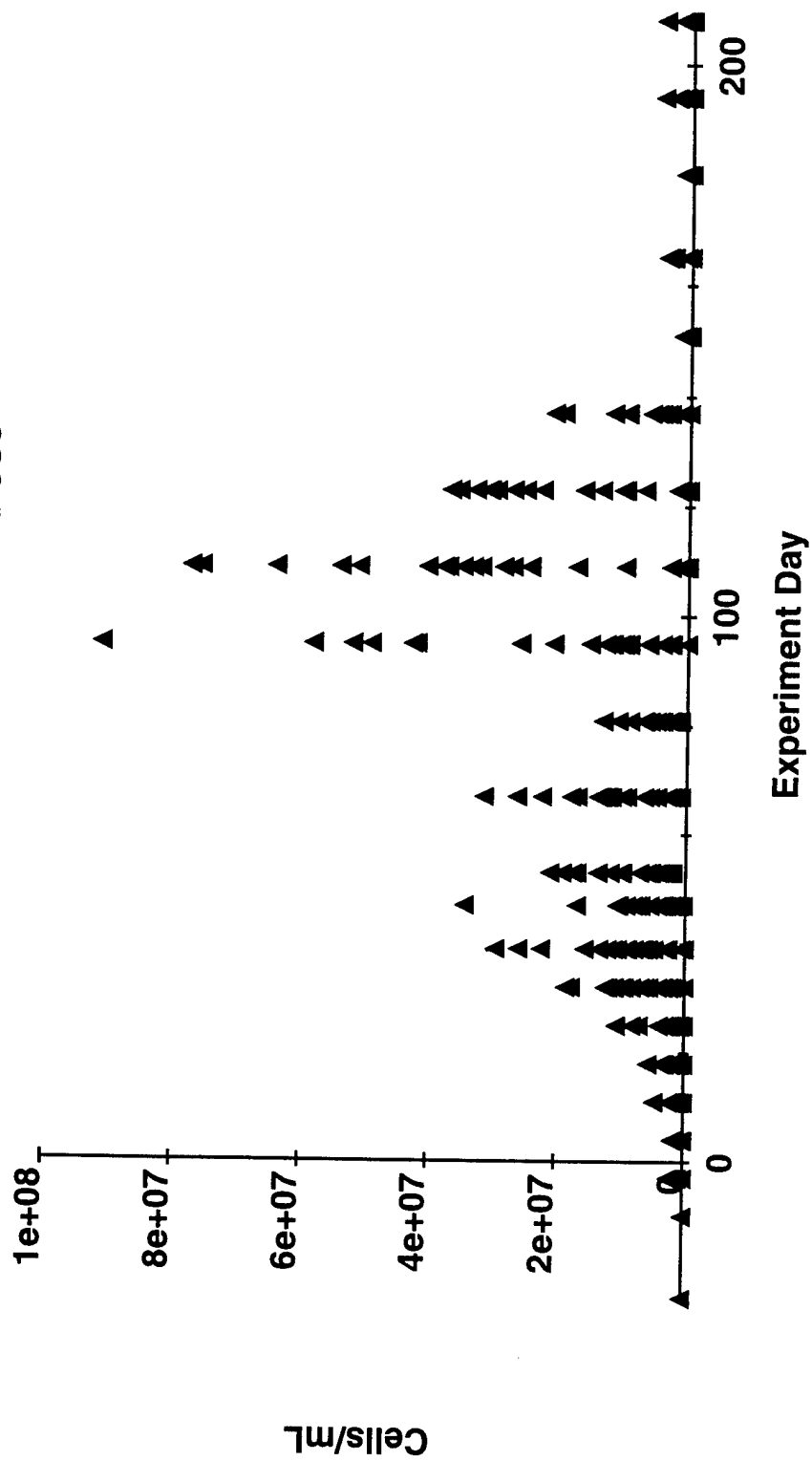
Figure 17d. Periphyton - Green Algae
Filamentous - Undosed
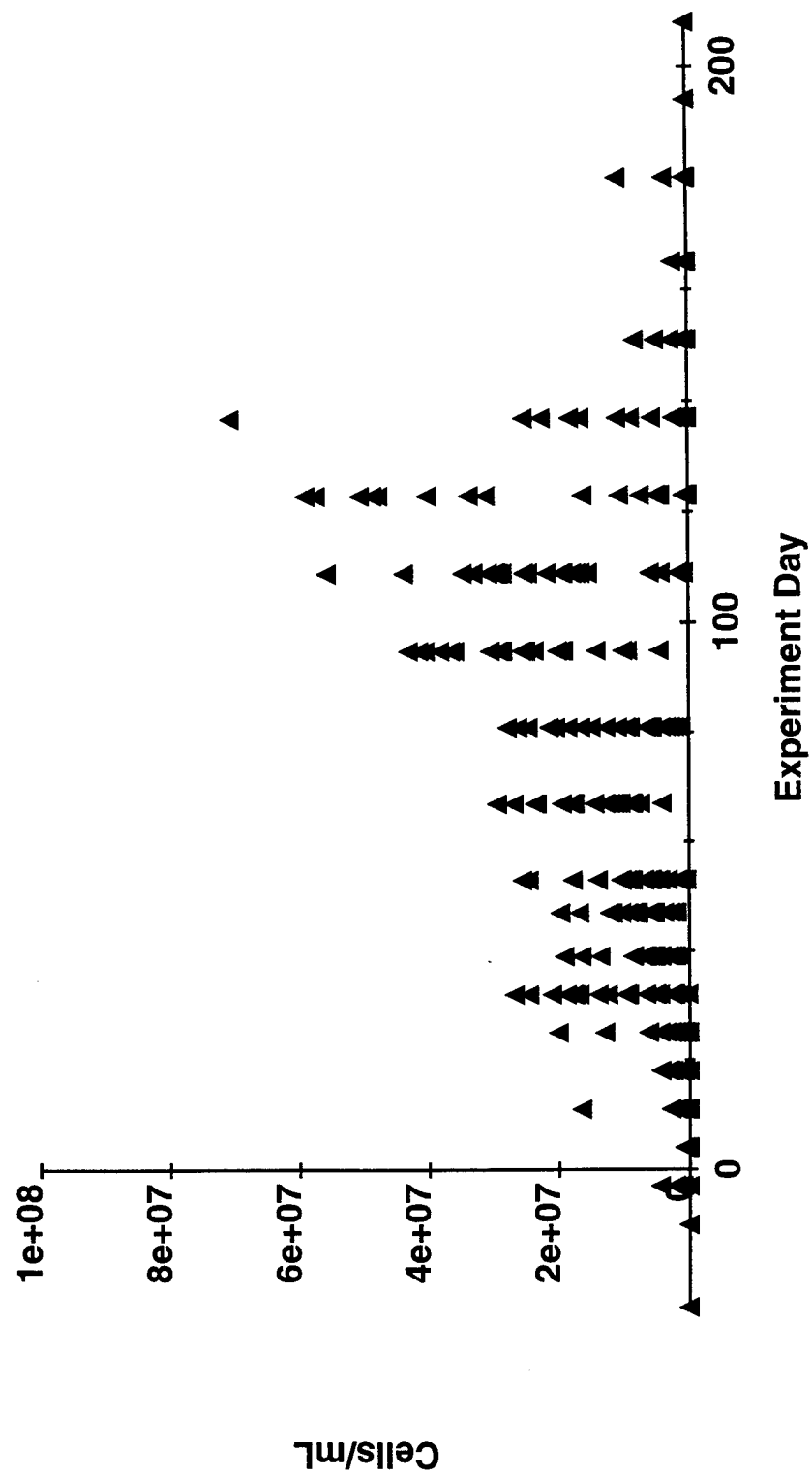
Figure 18a. Periphyton - Green Algae
Other, High Dose
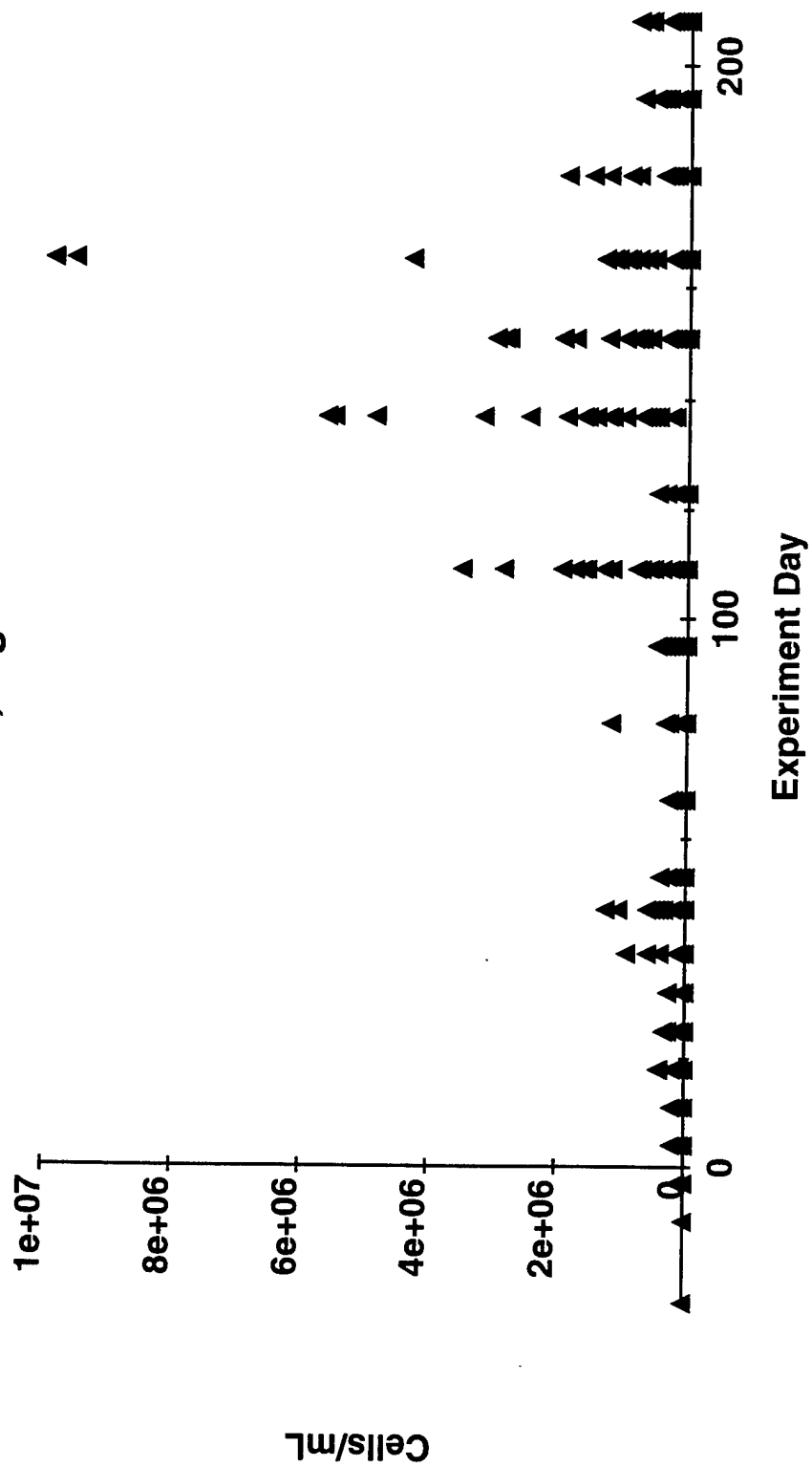
Figure 18b. Periphyton - Green Algae
Other - Mid-Dose
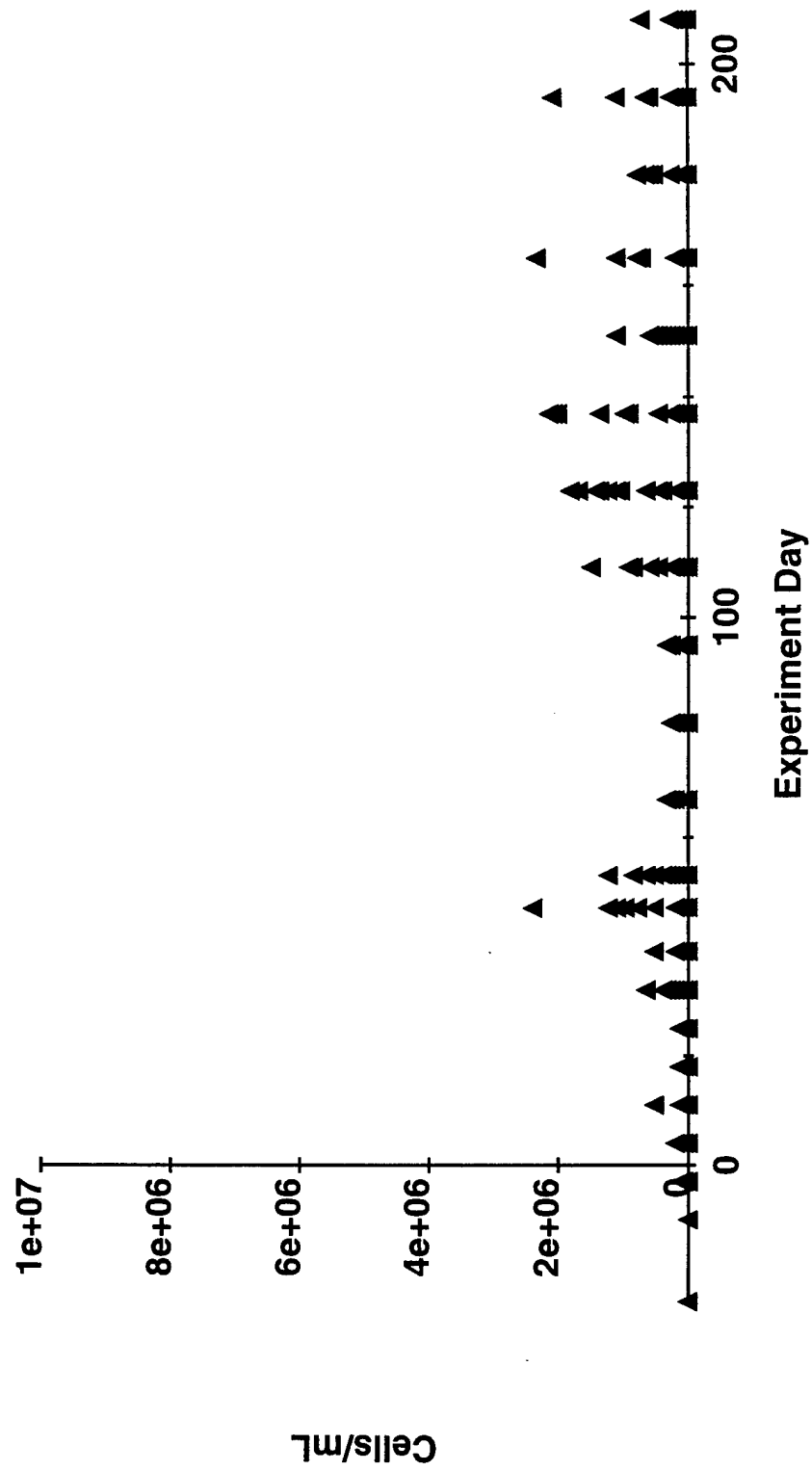
Figure 18c. Periphyton - Green Algae
Other - Low Dose
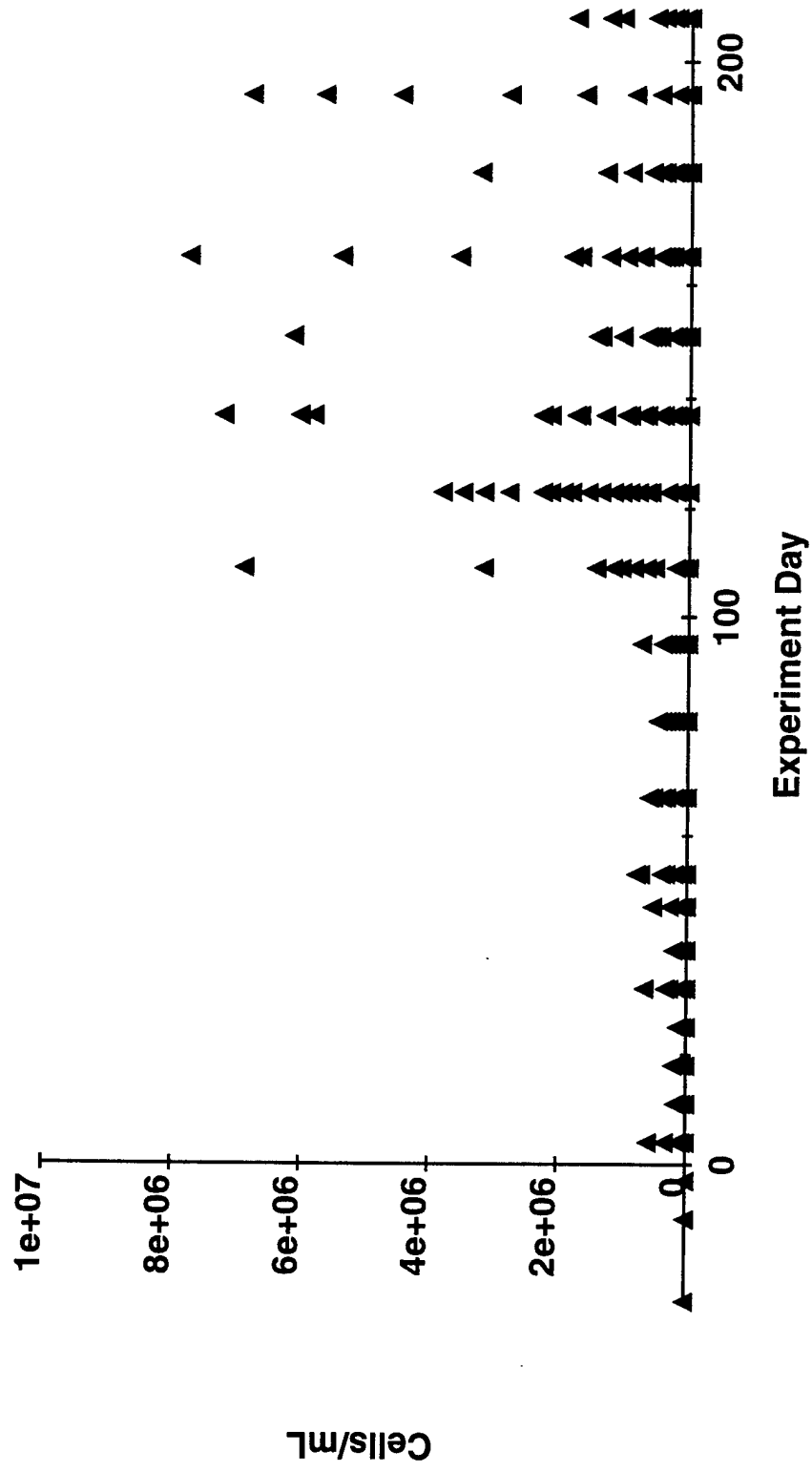
Figure 18d. Periphyton - Green Algae
Other - Undosed
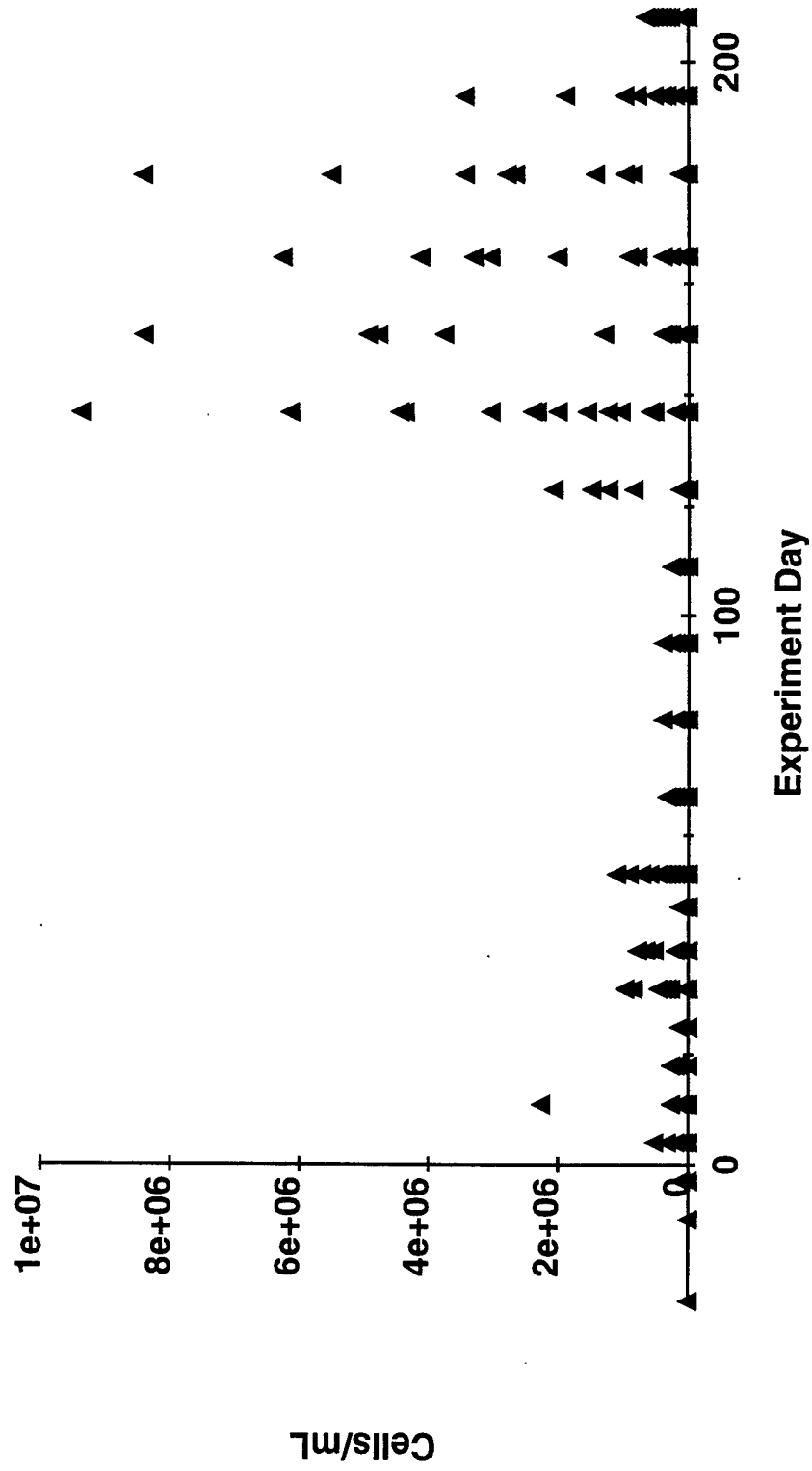
Figure 19a. Periphyton - Green Algae
Scenedesmus, High Dose
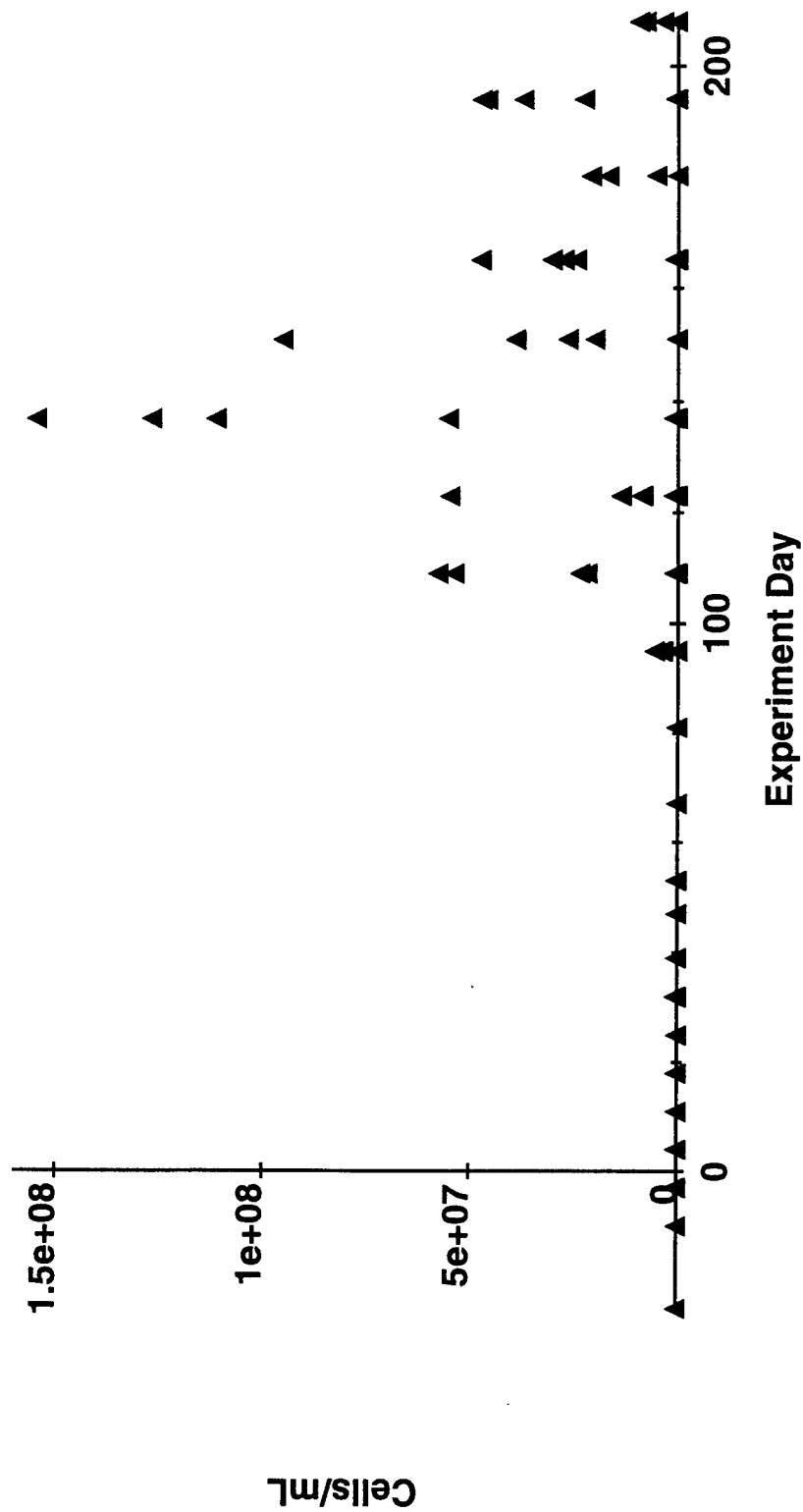
Figure 19b. Periphyton - Green Algae
Scenedesmus - Mid-Dose
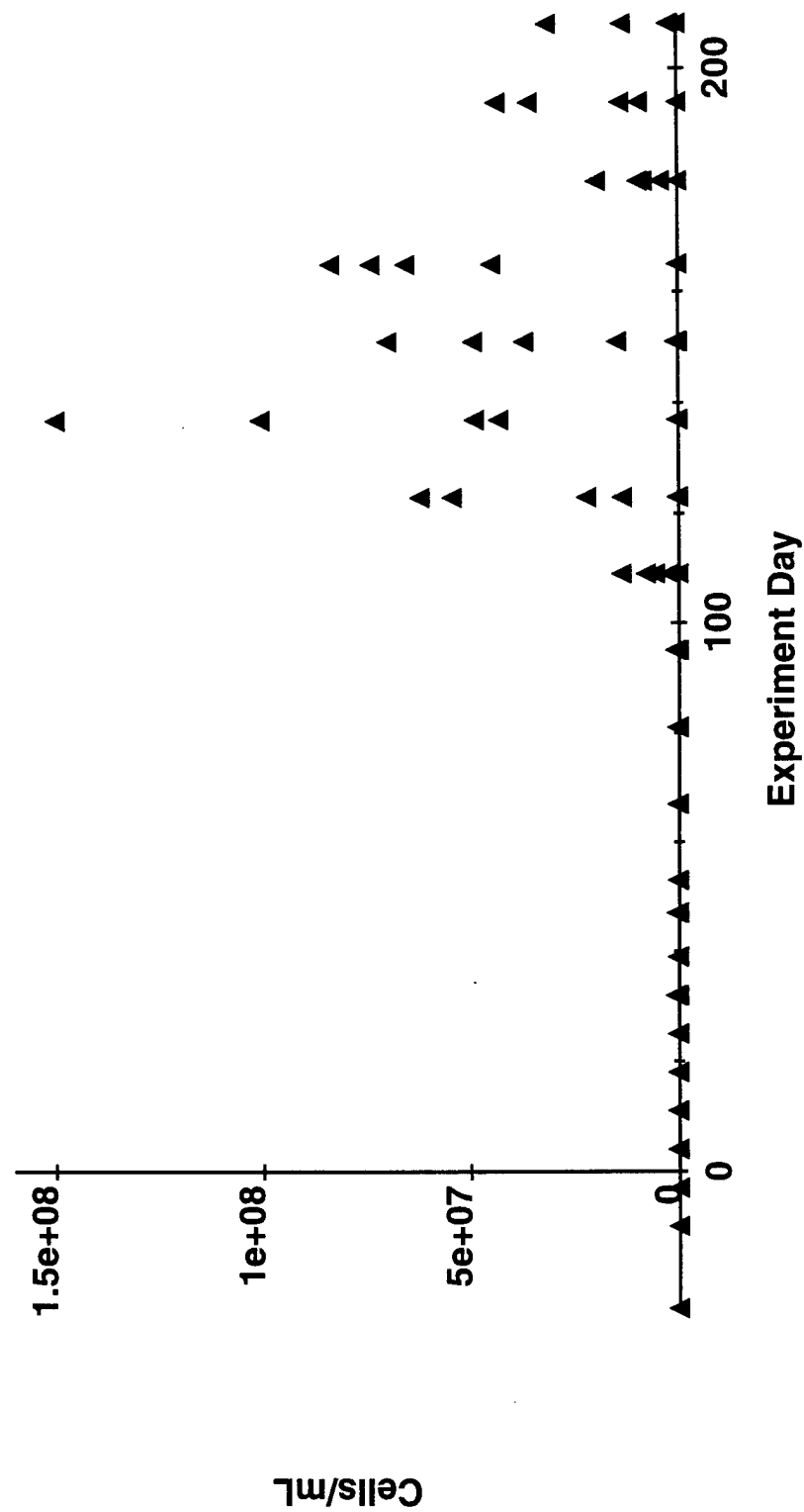
Figure 19c. Periphyton - Green Algae
Scenedesmus - Low Dose
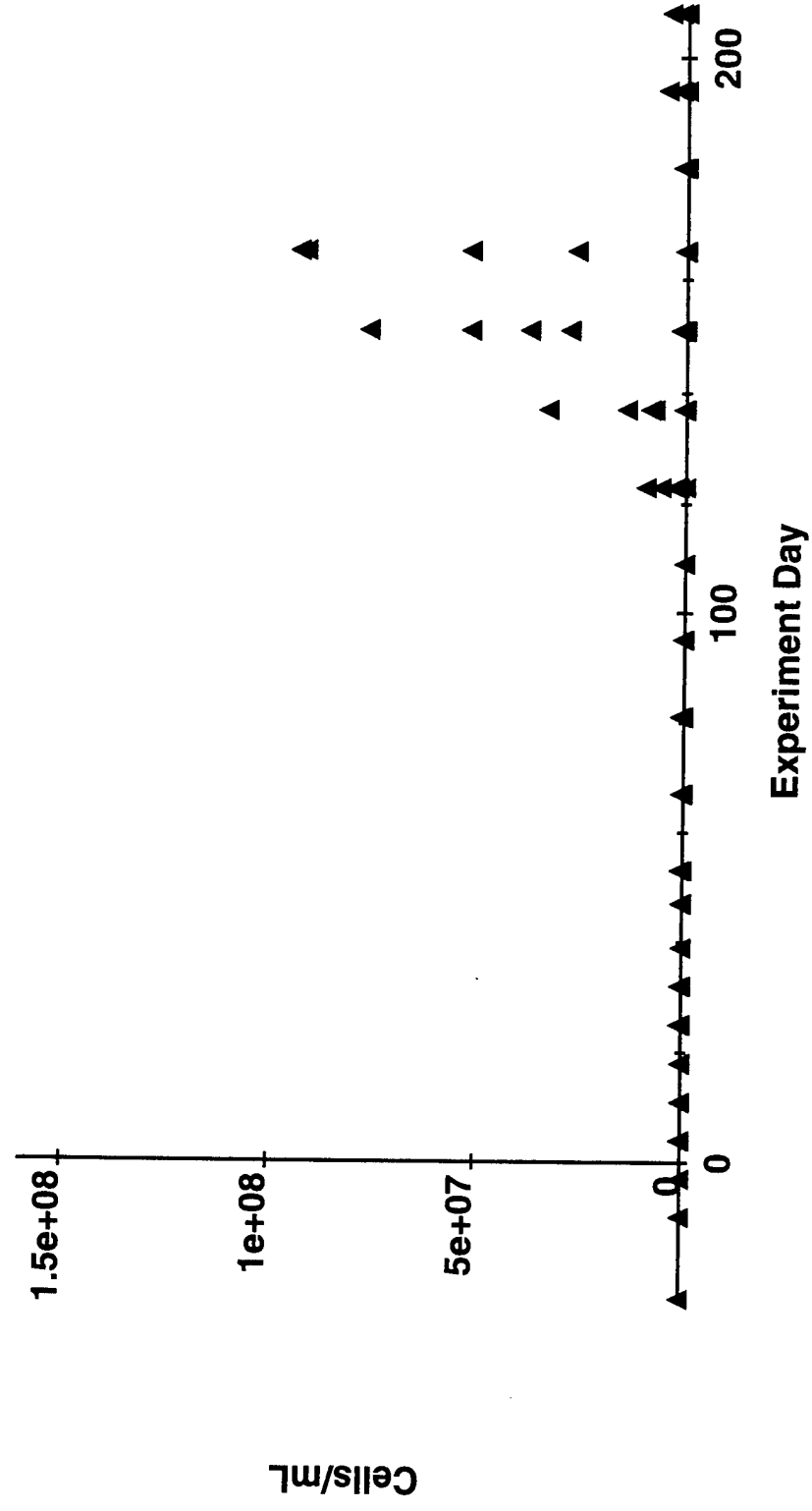
Figure 19d. Periphyton - Green Algae
Scenedesmus - Undosed
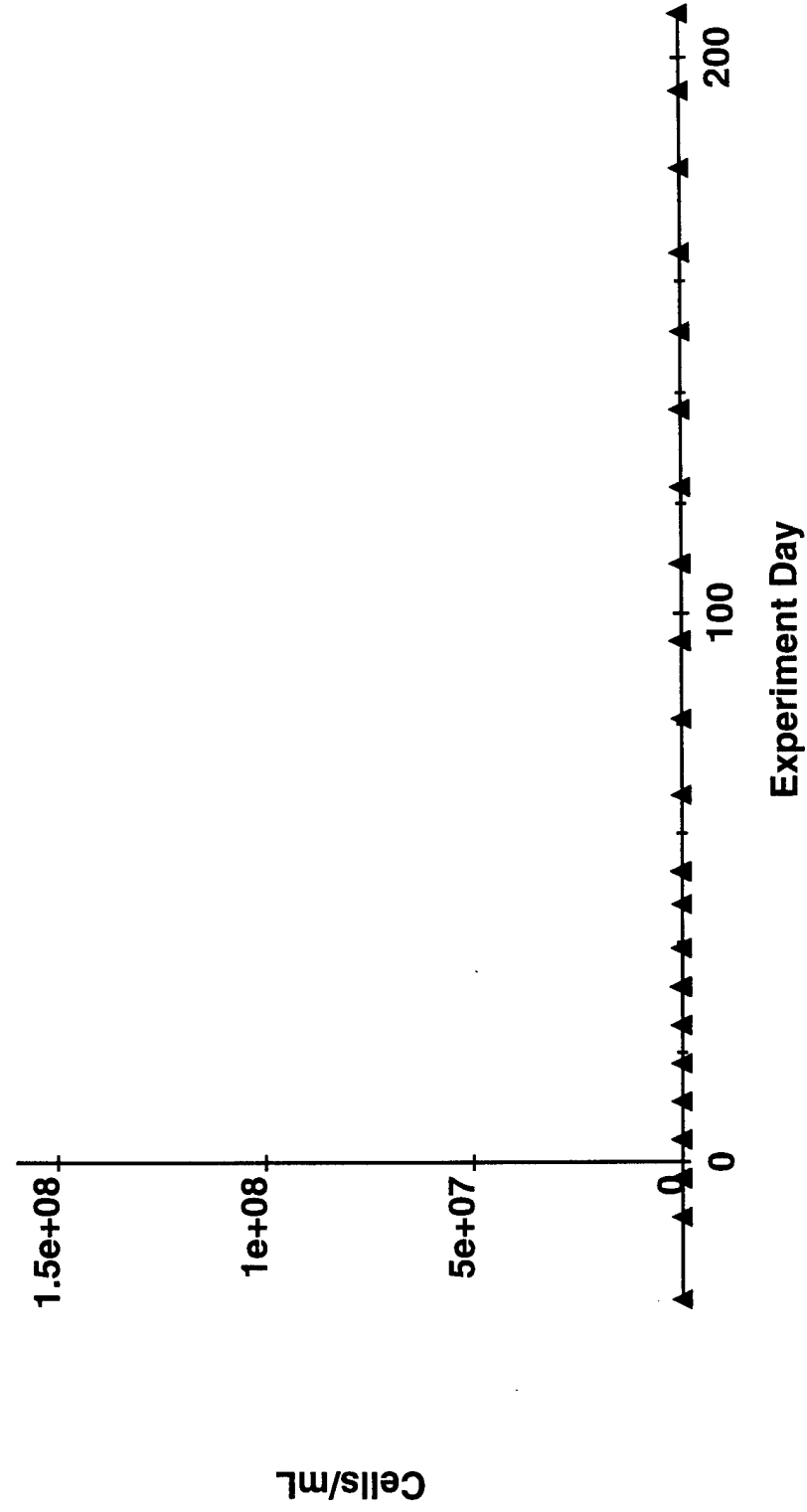
Figure 20a. Periphyton - Green Algae
Schroederia, High Dose
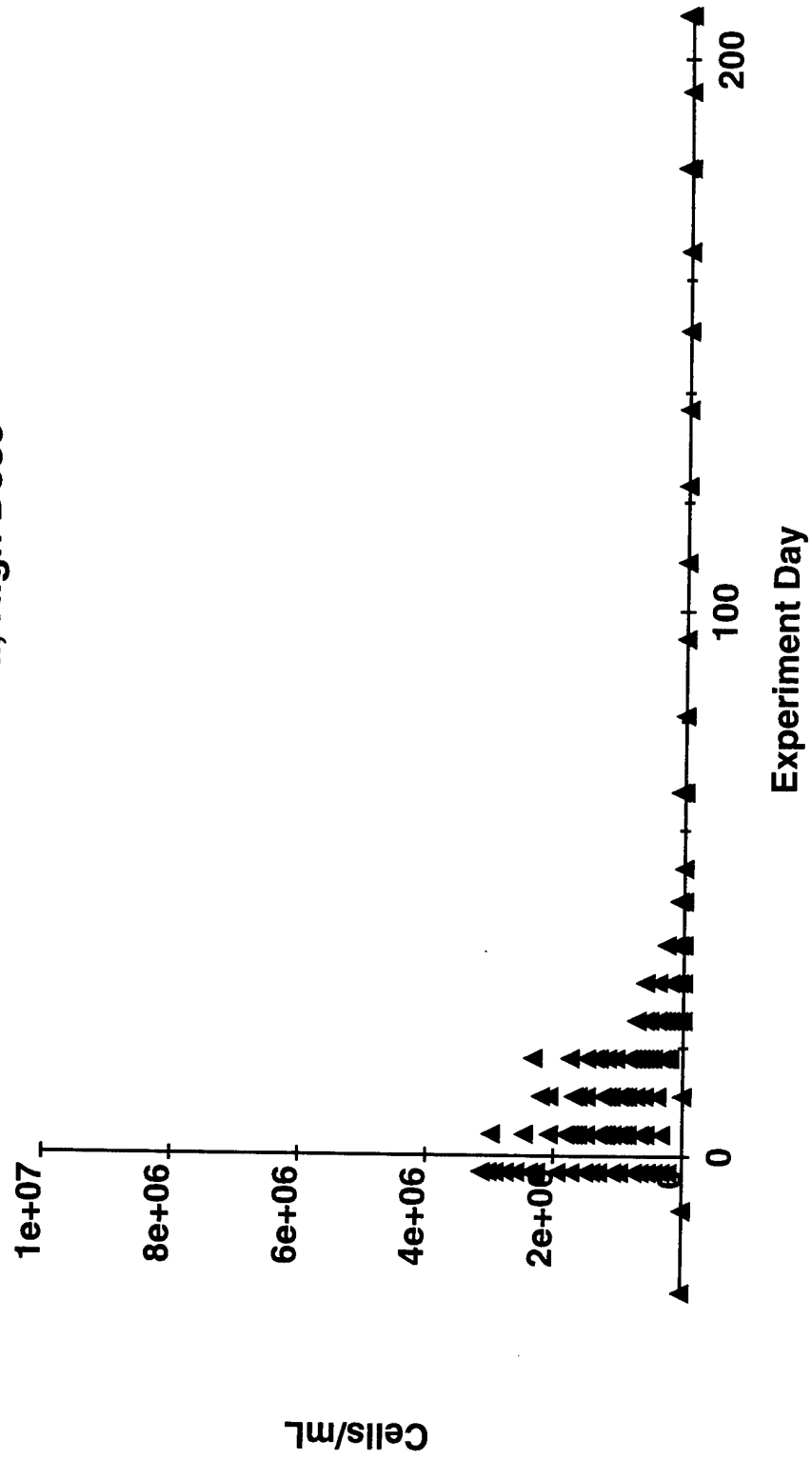
Figure 20b. Periphyton - Green Algae
Schroederia - Mid-Dose
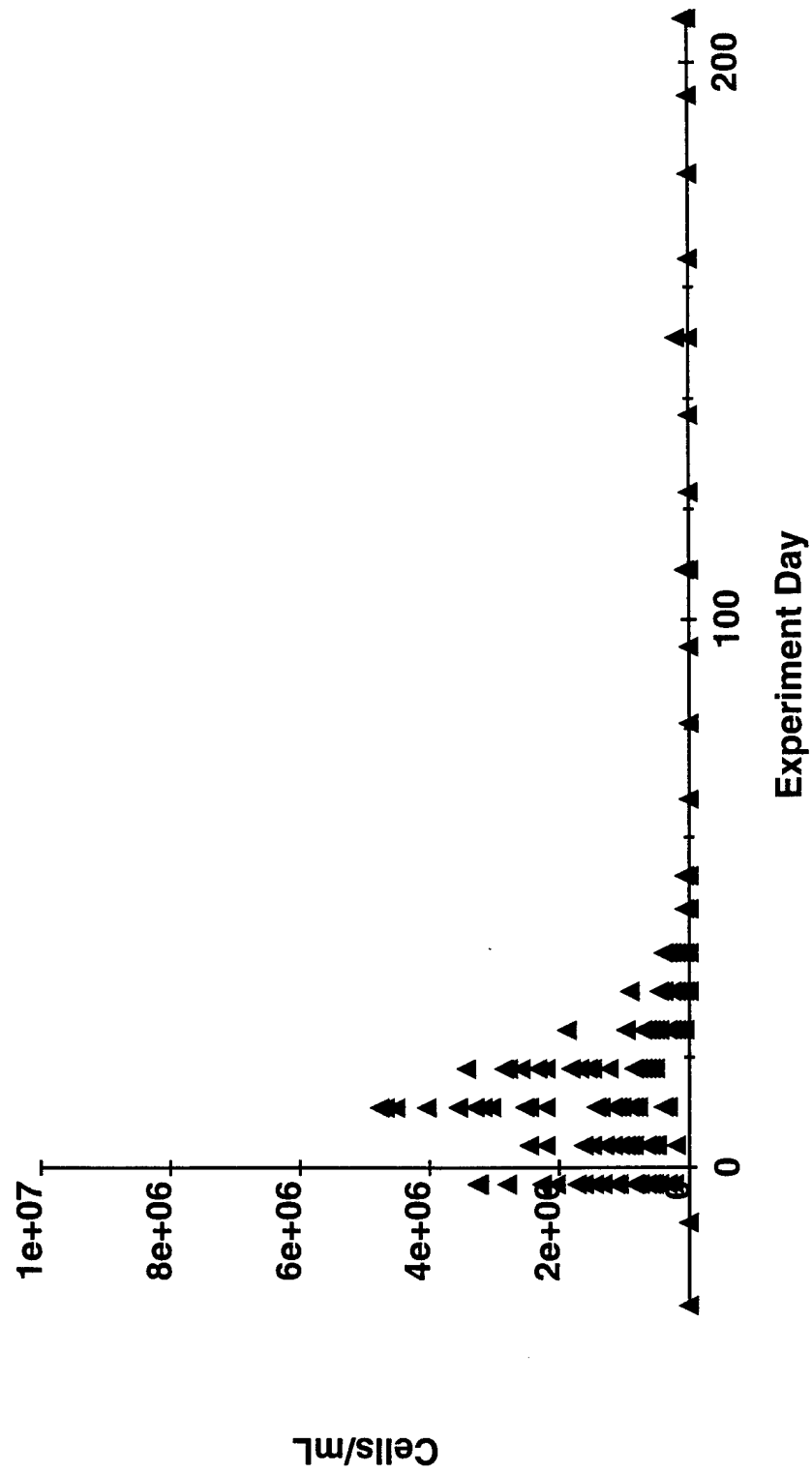
Figure 20c. Periphyton - Green Algae
Schroederia - Low Dose
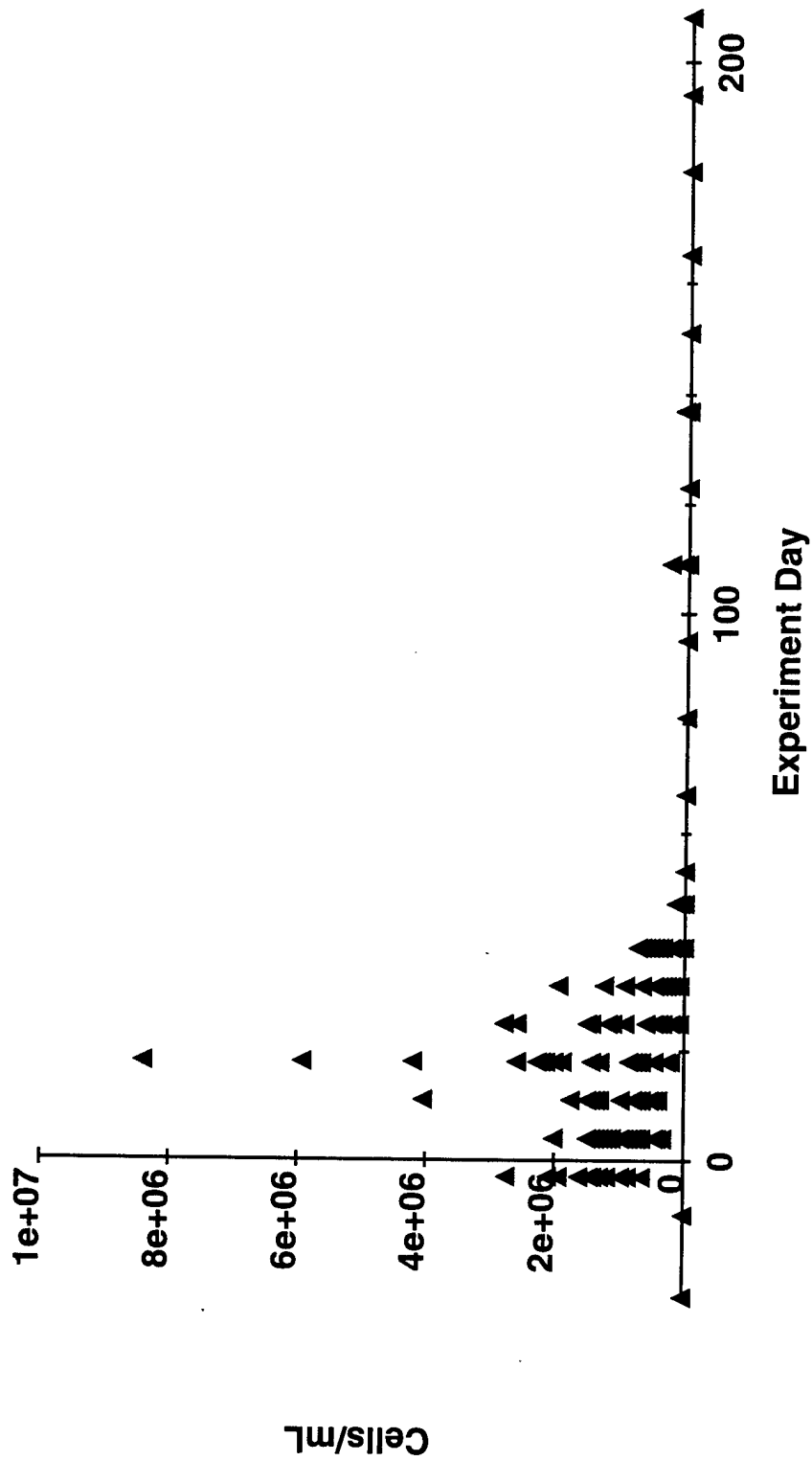
Figure 20d. Periphyton - Green Algae
Schroederia - Undosed
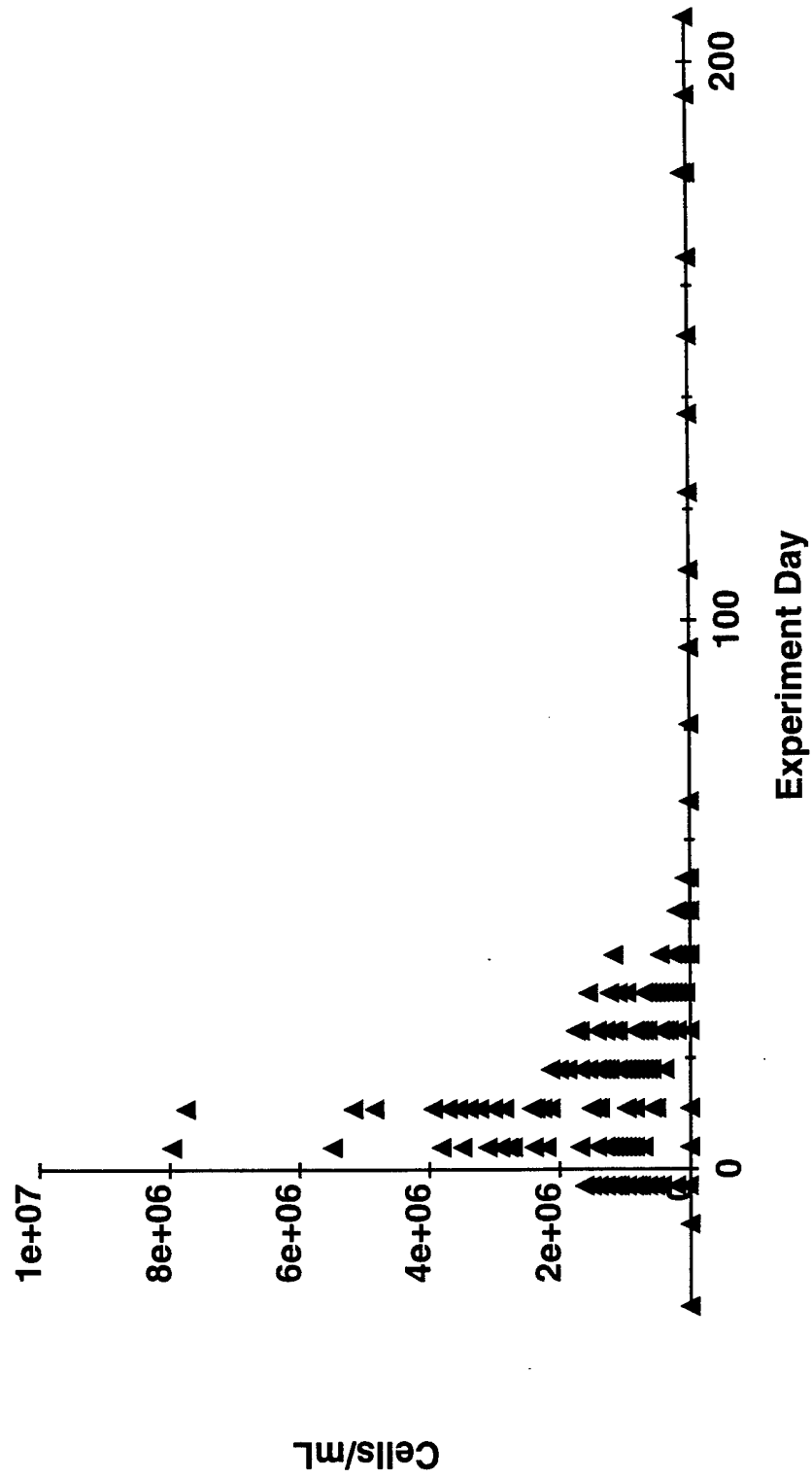
Figure 21a. Periphyton - Green Algae
Selenastrum, High Dose
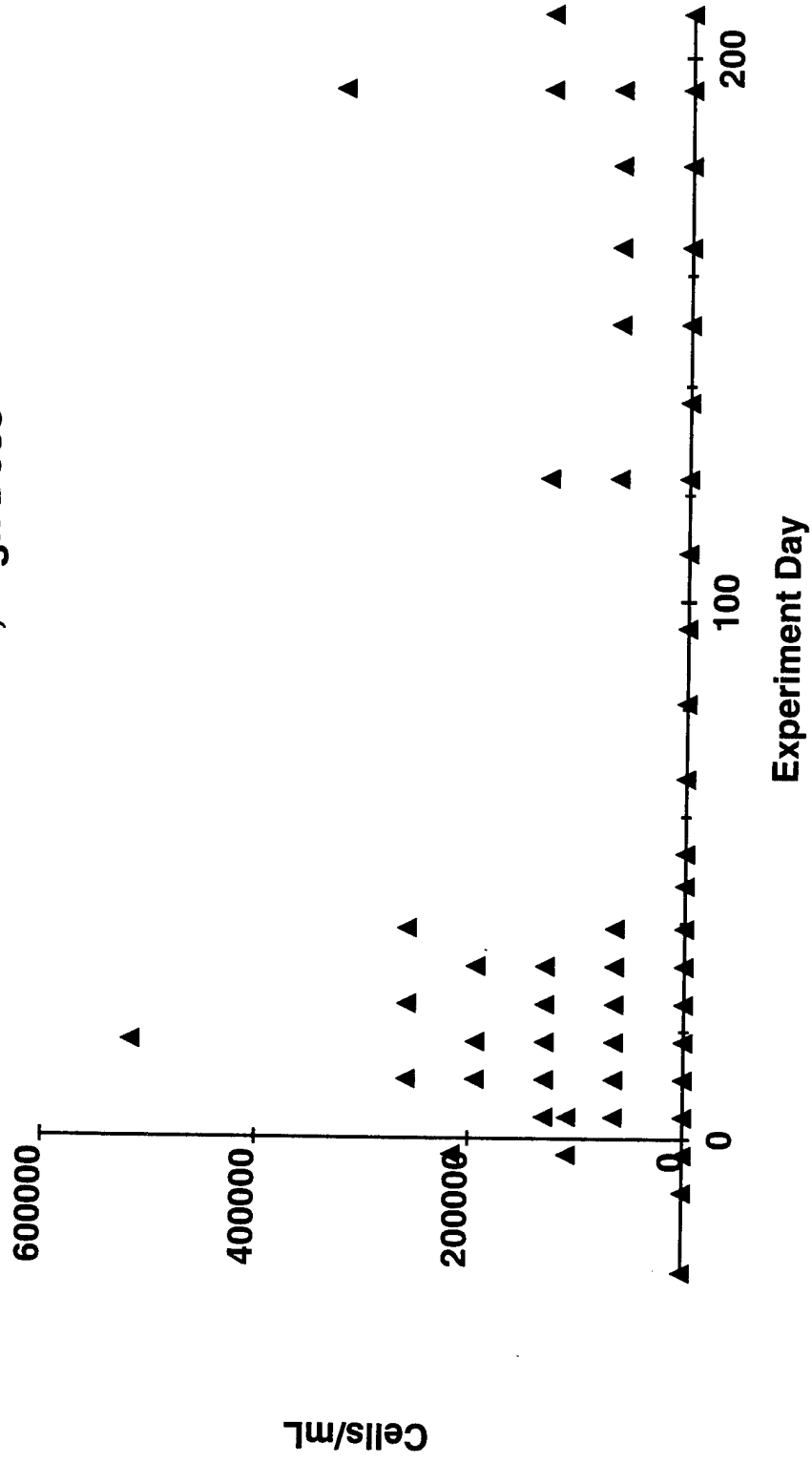
Figure 21b. Periphyton - Green Algae
Selenastrum - Mid-Dose
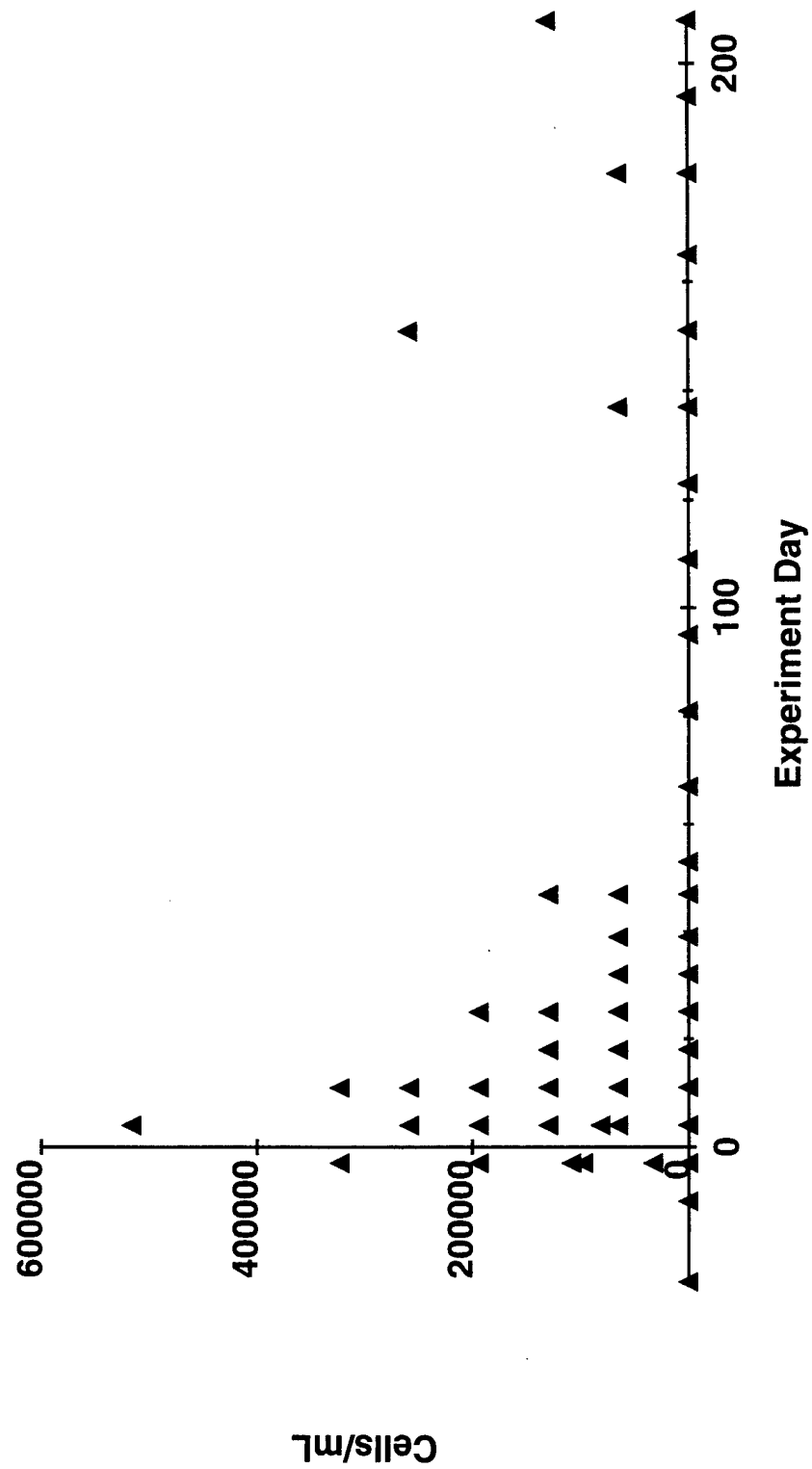
Figure 21c. Periphyton - Green Algae
Selenastrum - Low Dose
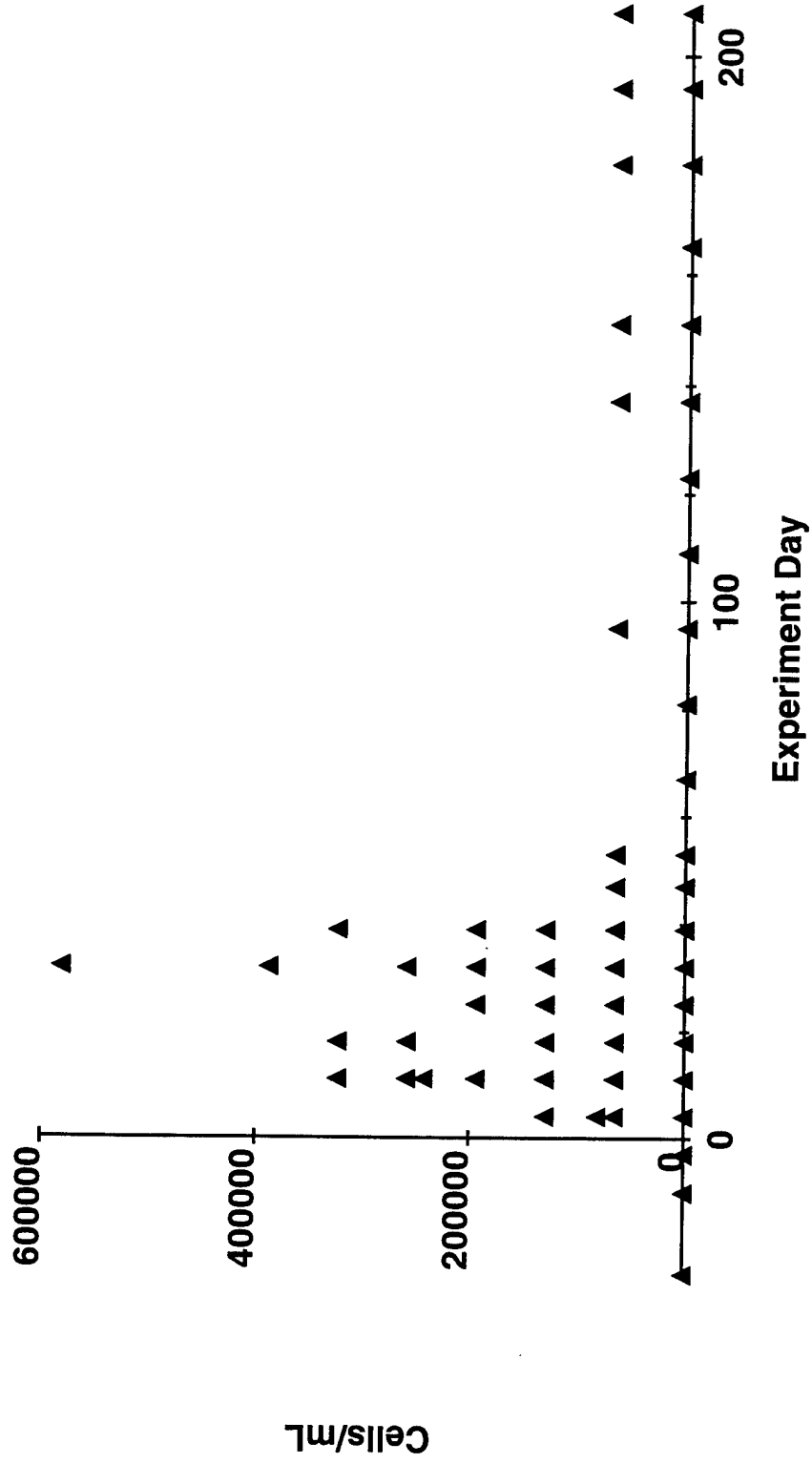
Figure 21d. Periphyton - Green Algae
Selenastrum - Undosed
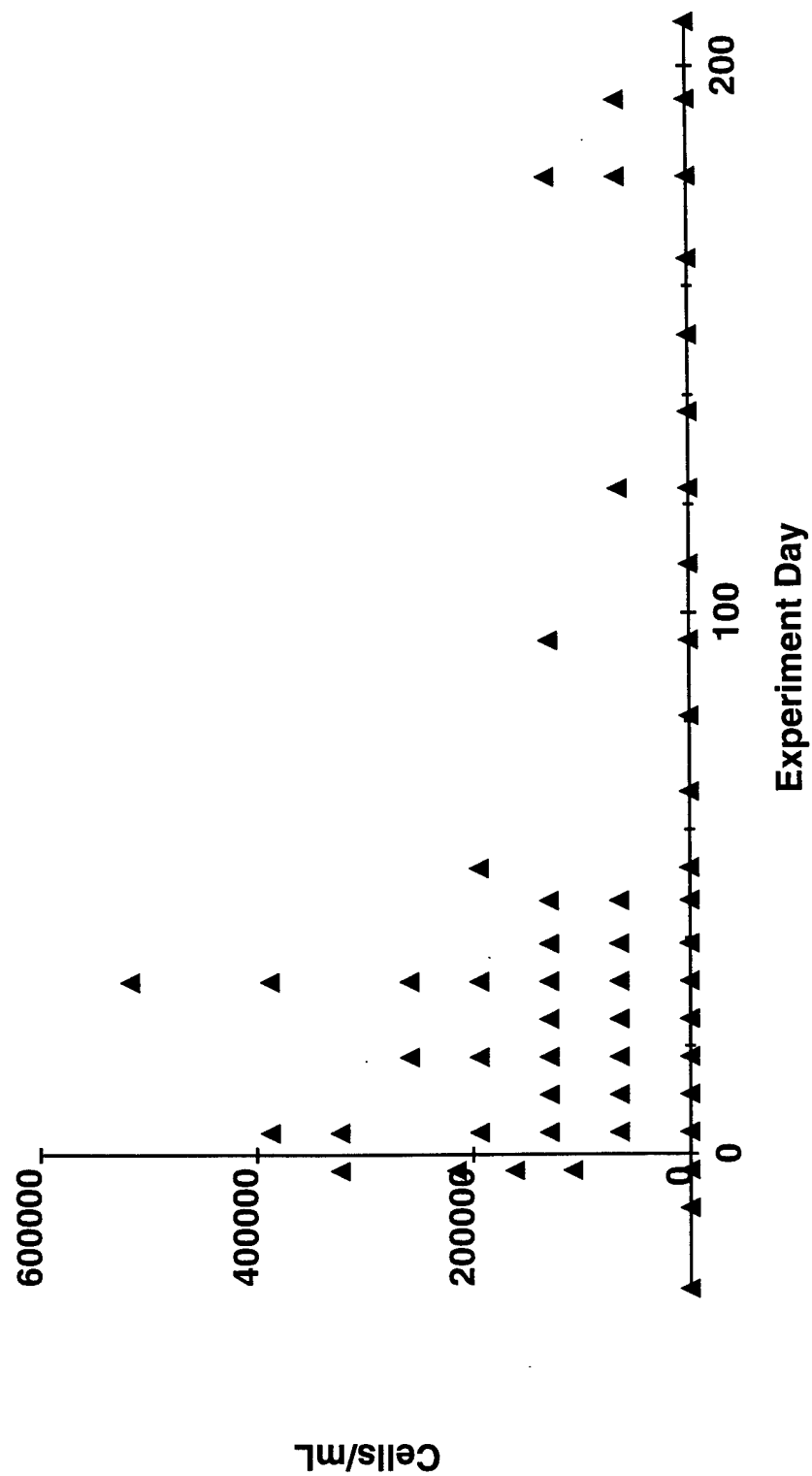
Figure 22a. Periphyton - Miscellaneous
Amoebas, High Dose
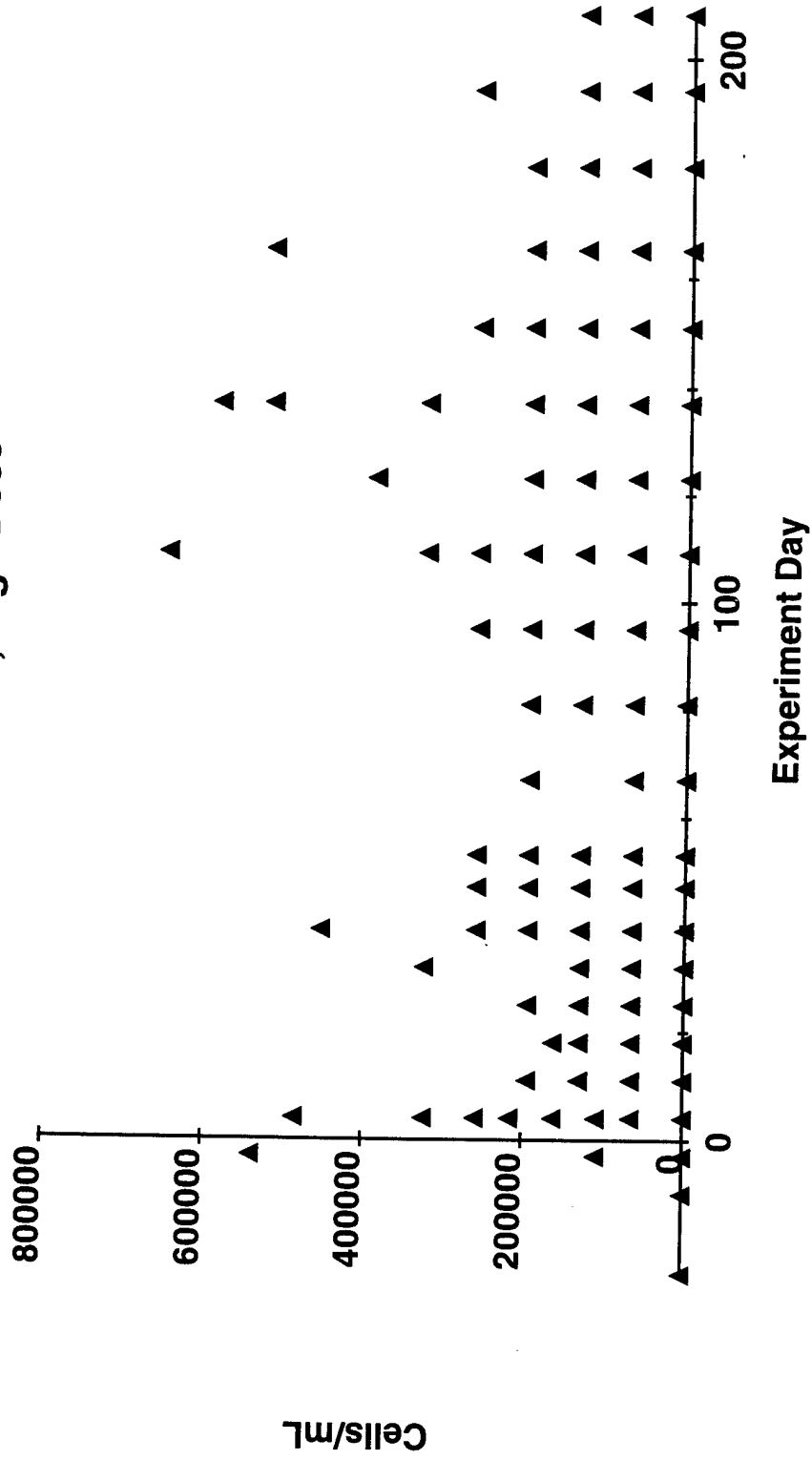
Figure 22b. Periphyton - Miscellaneous
Amoebas - Mid-Dose

Cells/mL

Experiment Day
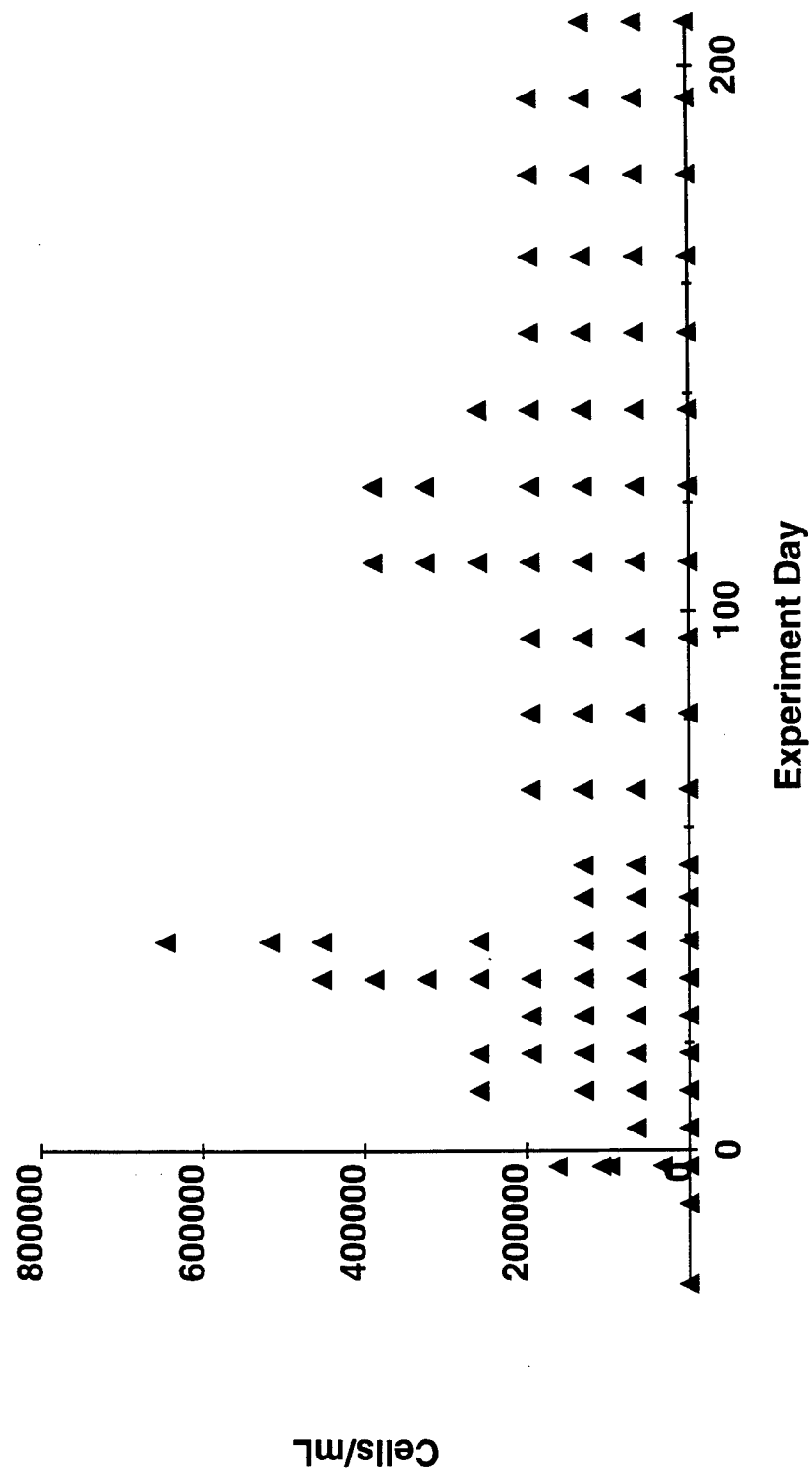
Figure 22c. Periphyton - Miscellaneous
Amoebas - Low Dose
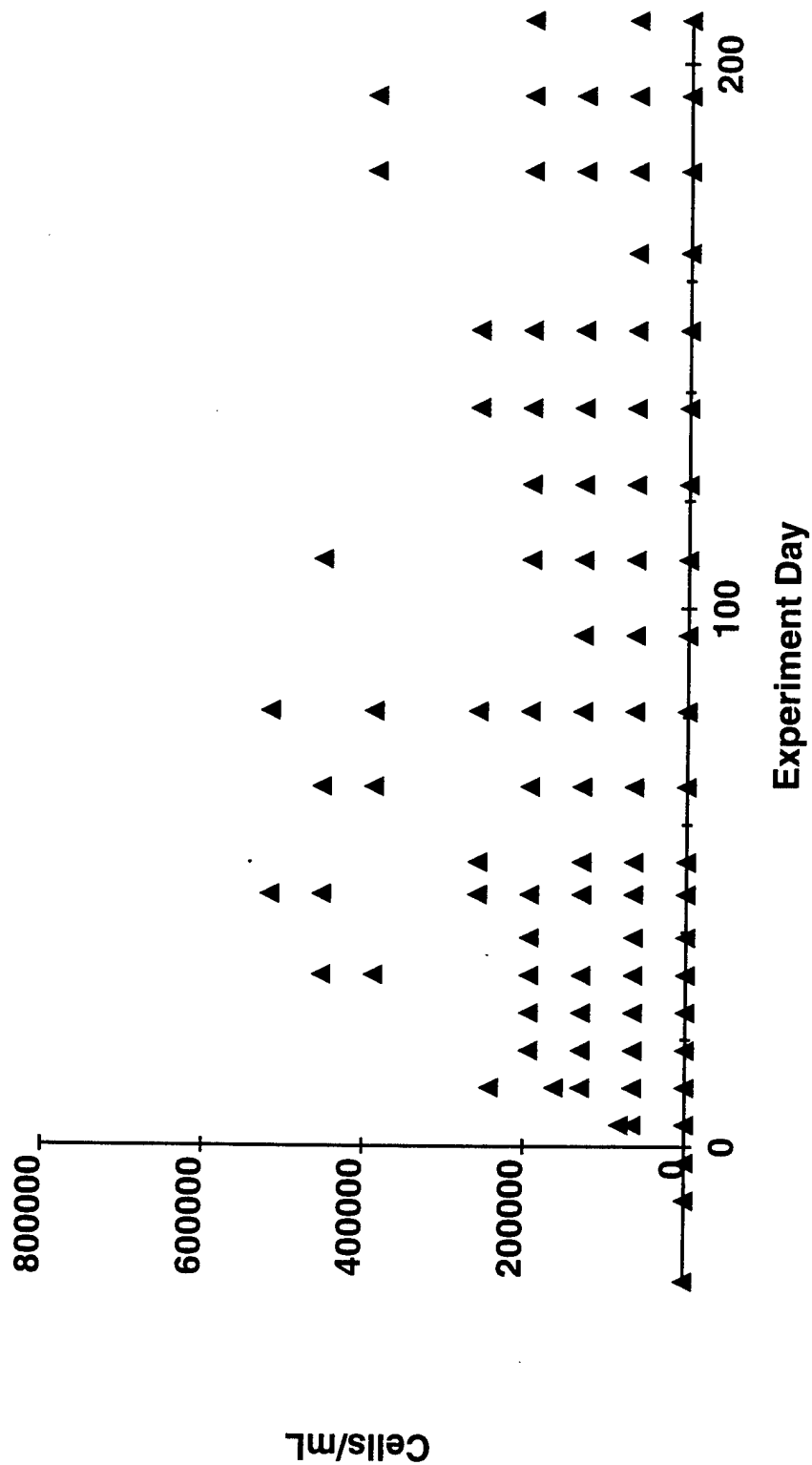
Figure 22d. Periphyton - Miscellaneous
Amoebas - Undosed
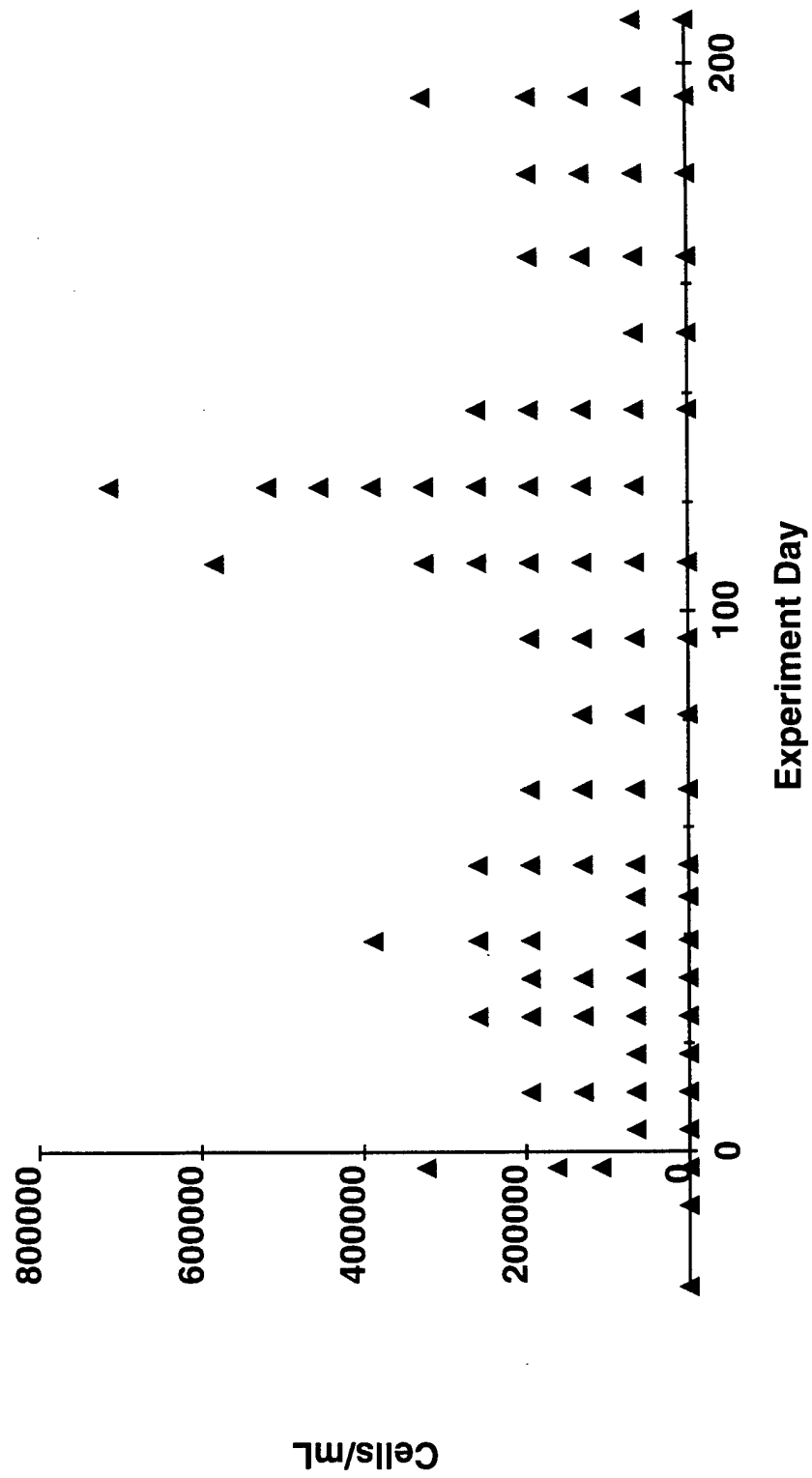
Figure 23a. Periphyton - Miscellaneous
Rotifers, High Dose
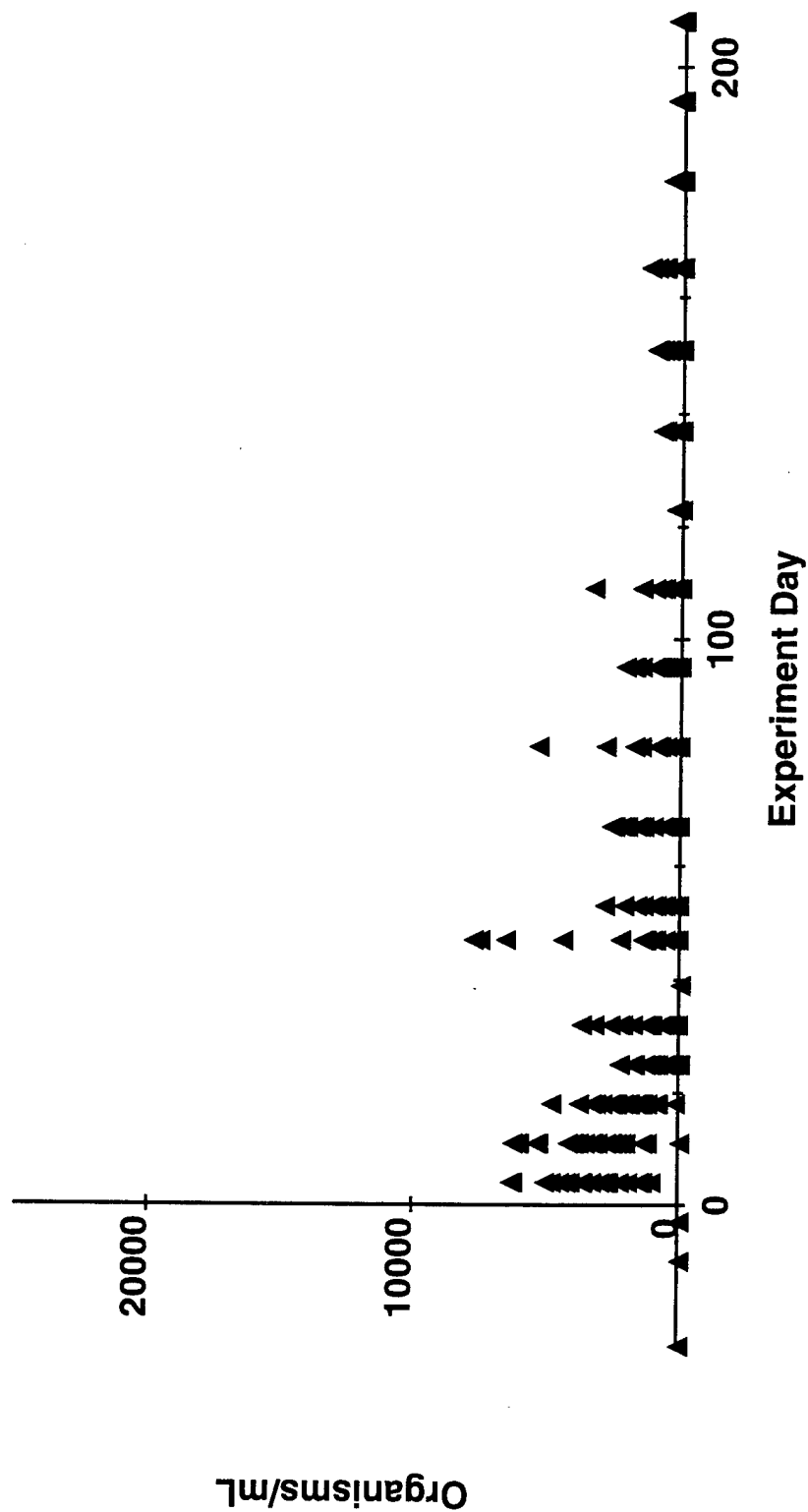
Figure 23b. Periphyton - Miscellaneous
Rotifers - Mid-Dose
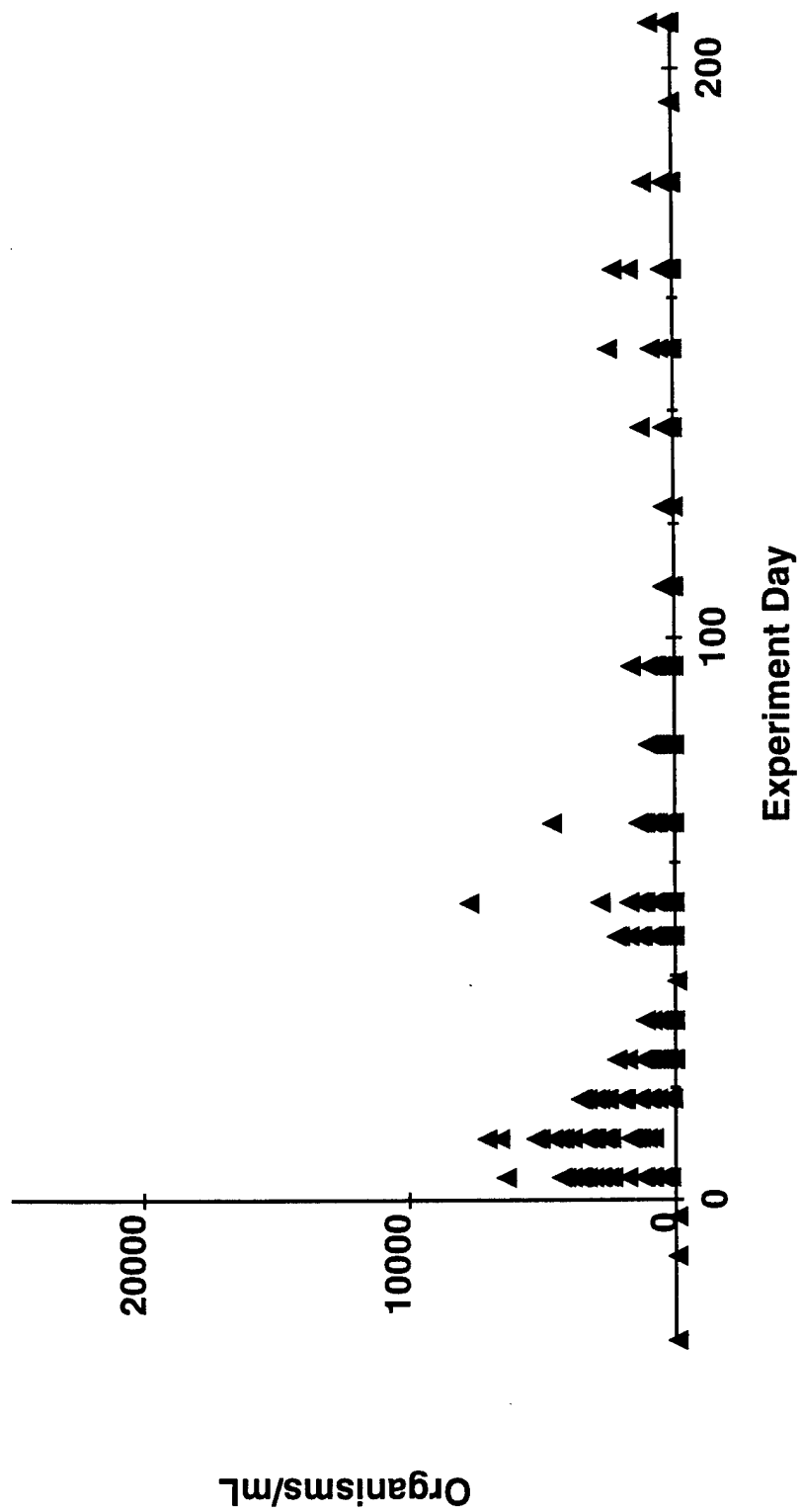
Figure 23c. Periphyton - Miscellaneous
Rotifers - Low Dose
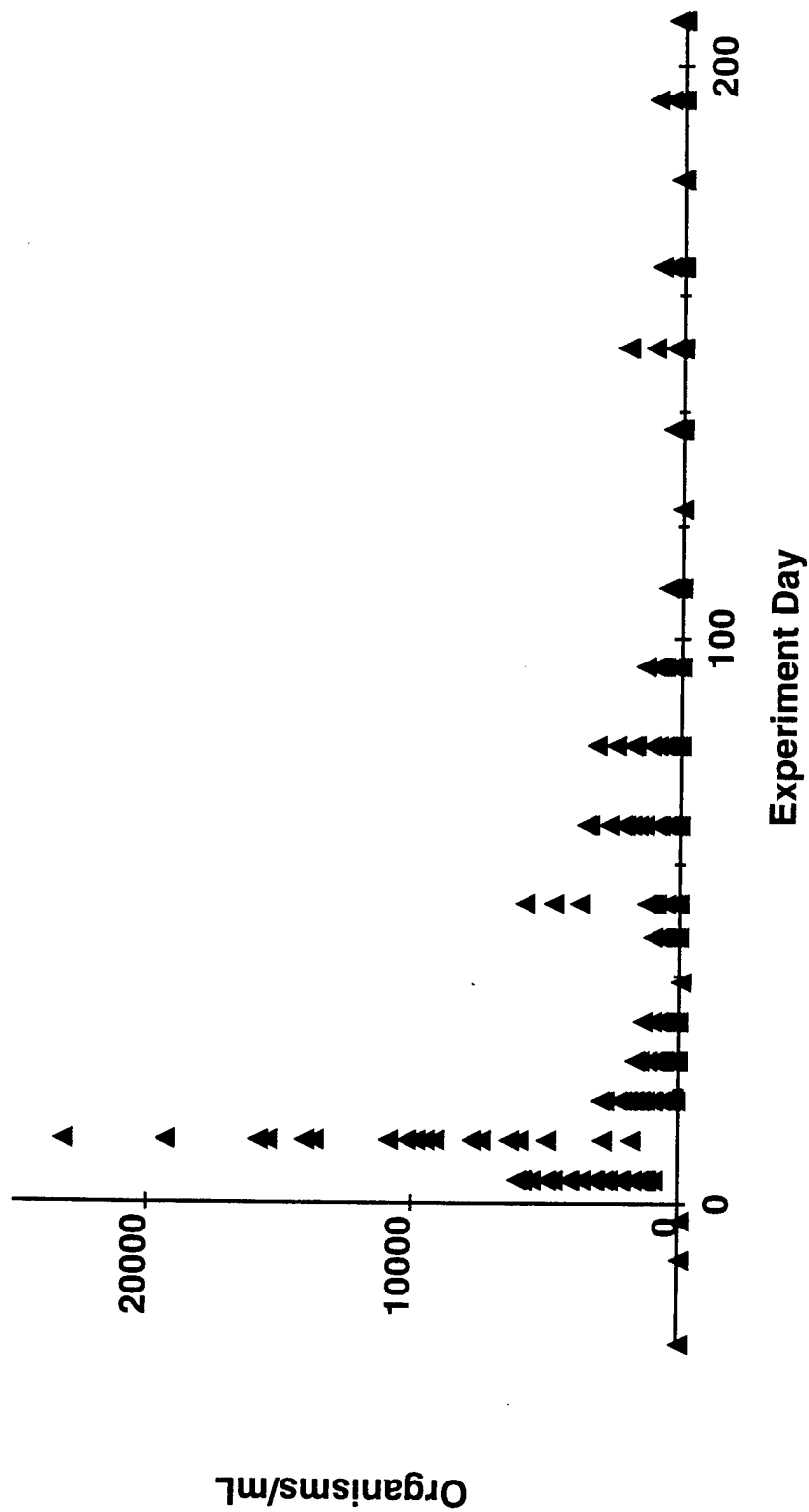
Figure 23d. Periphyton - Miscellaneous
Rotifers - Undosed
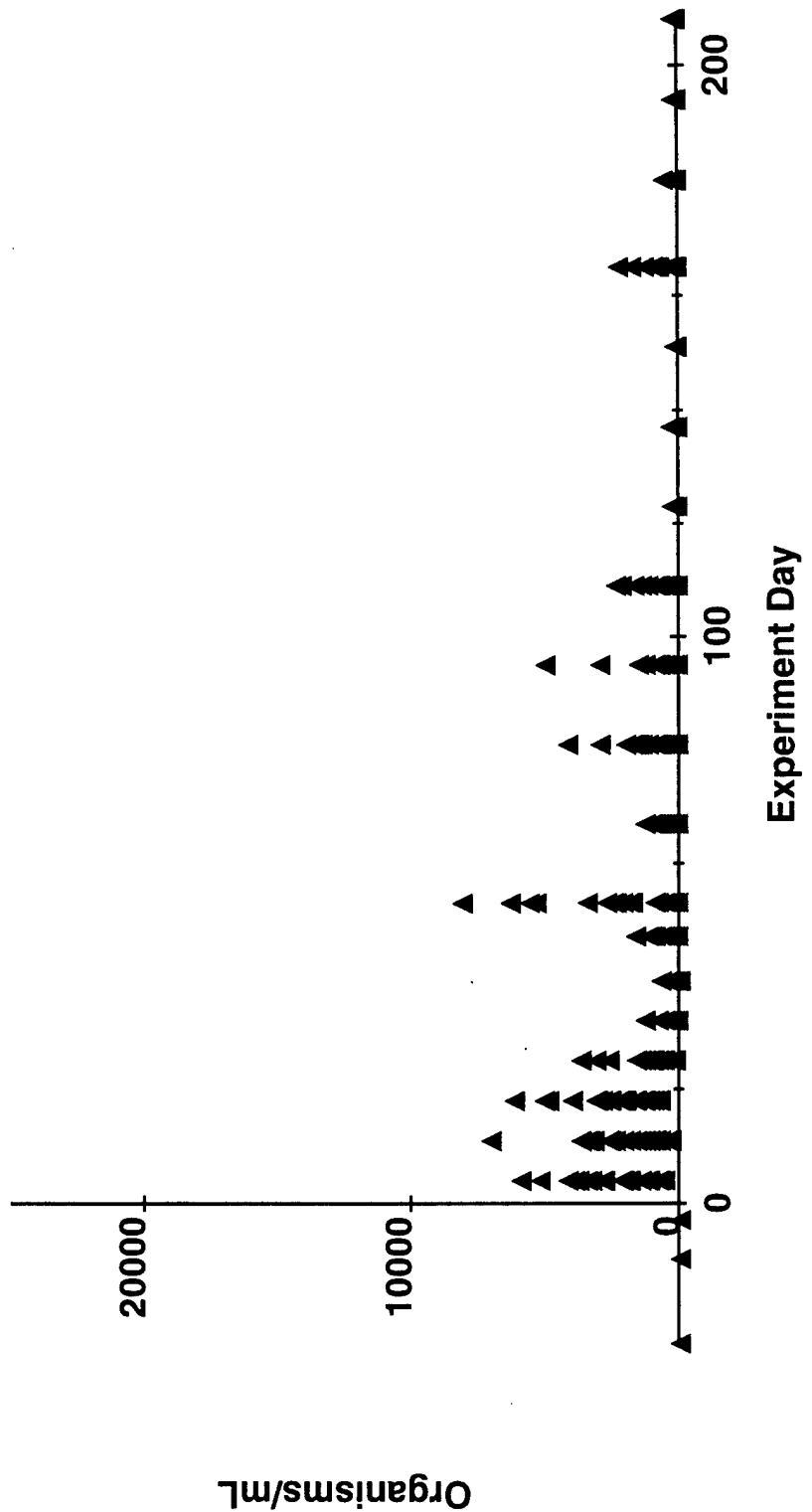
Figure 24a. Periphyton
Ash-Free Dry Weight, High Dose
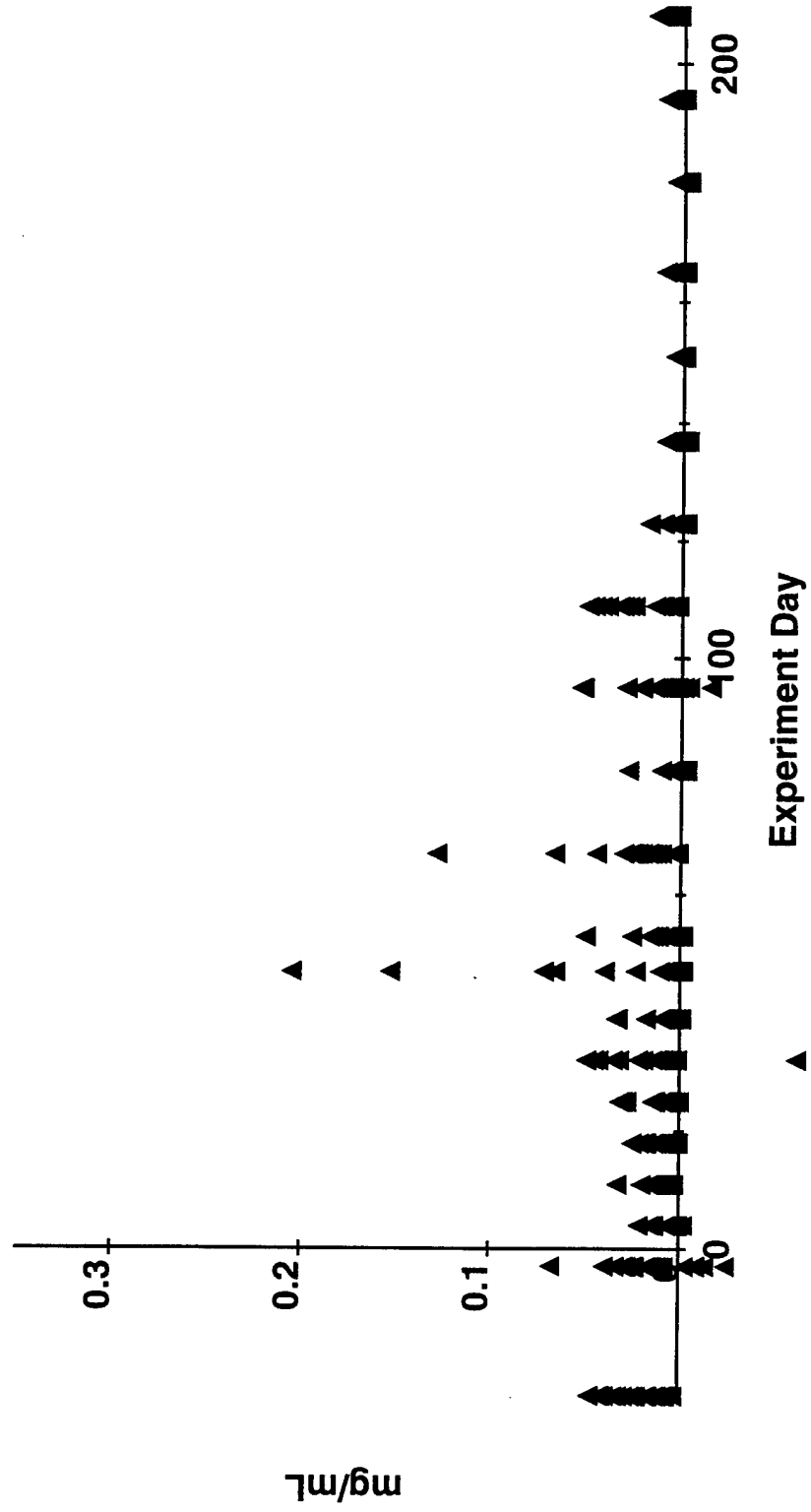
Figure 24b. Periphyton
Ash-Free Dry Weight - Mid-Dose
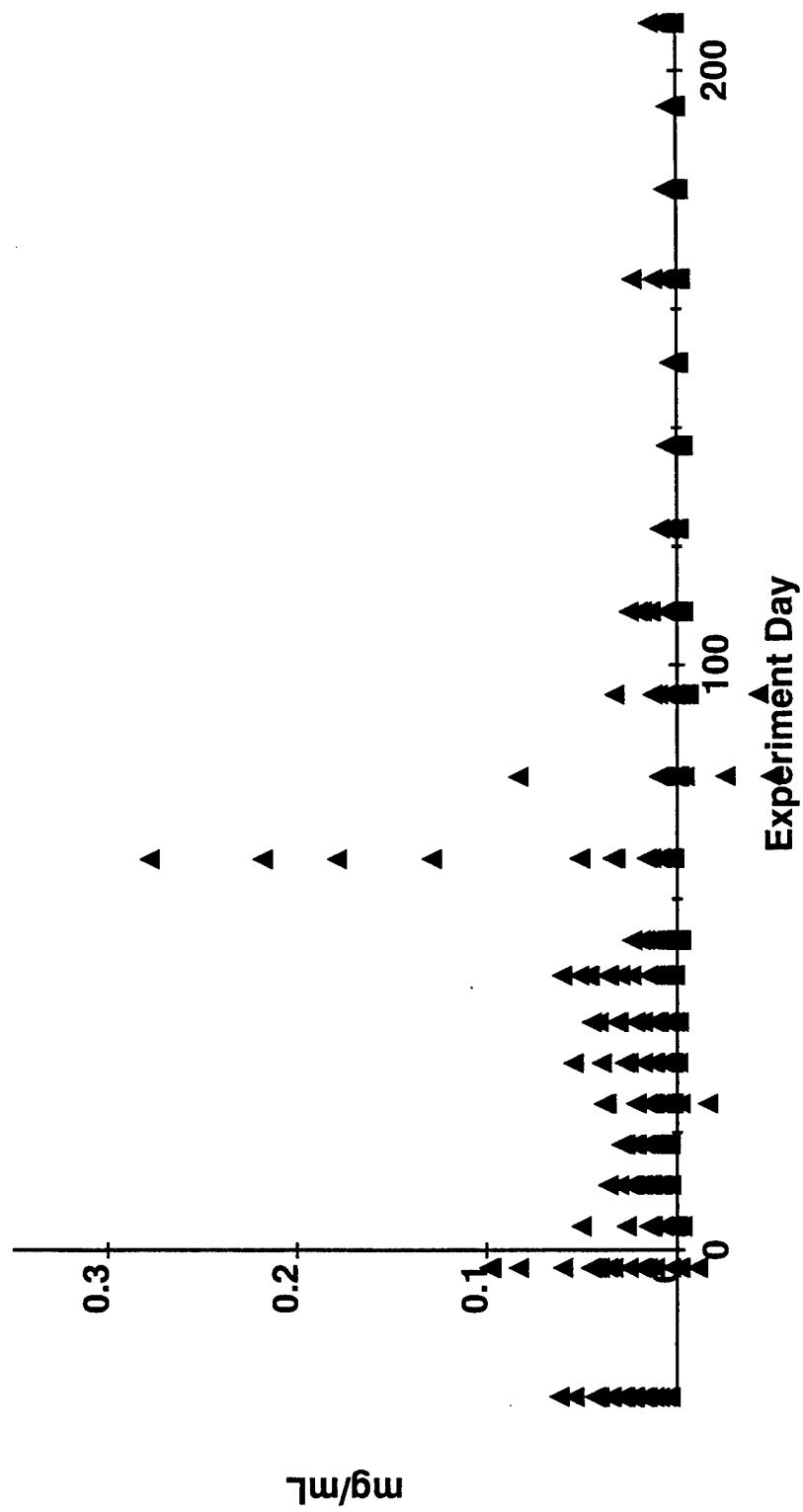
Figure 24c. Periphyton
Ash-Free Dry Weight - Low Dose
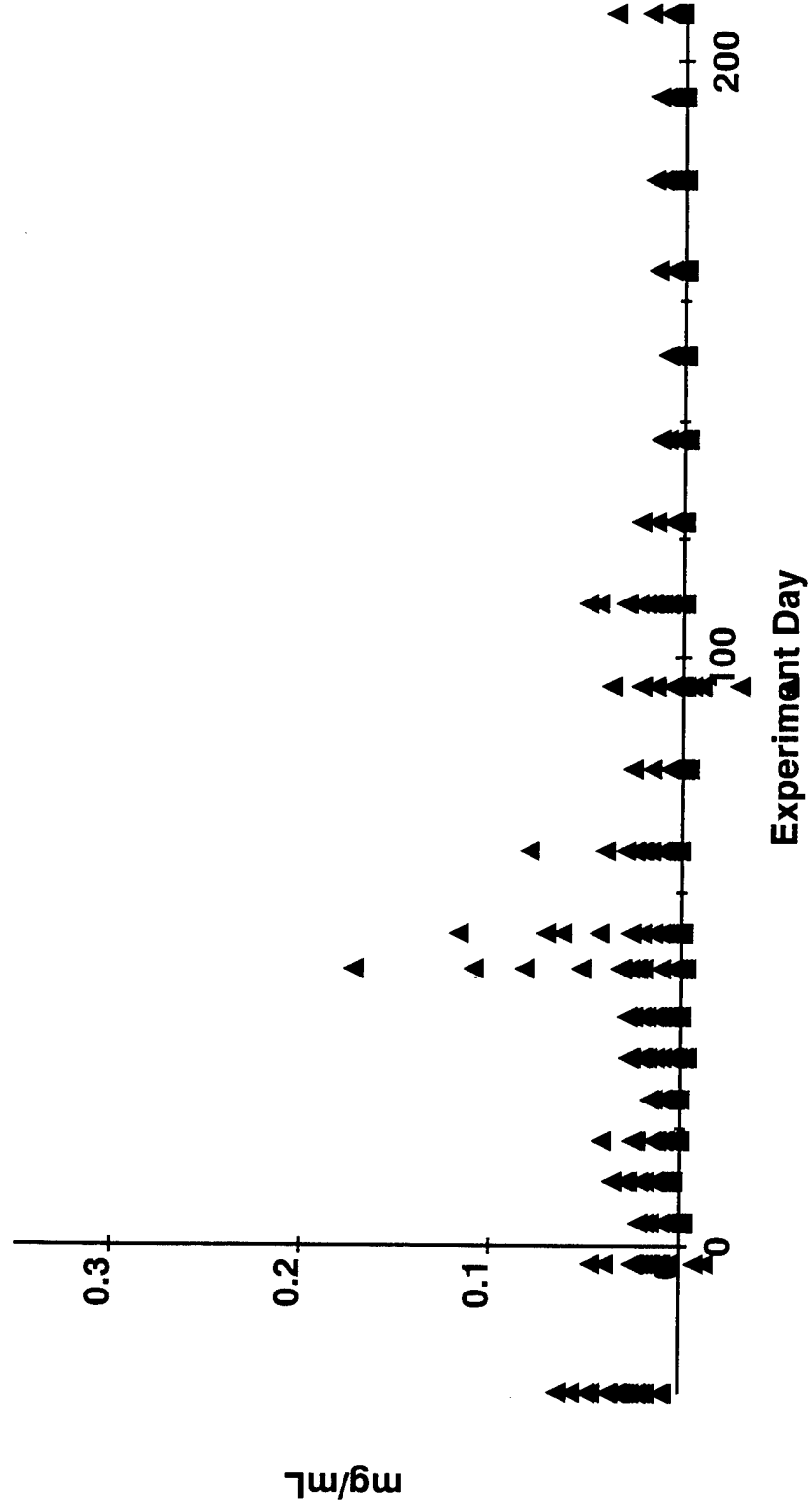
Figure 24d. Periphyton
Ash-Free Dry Weight - Undosed
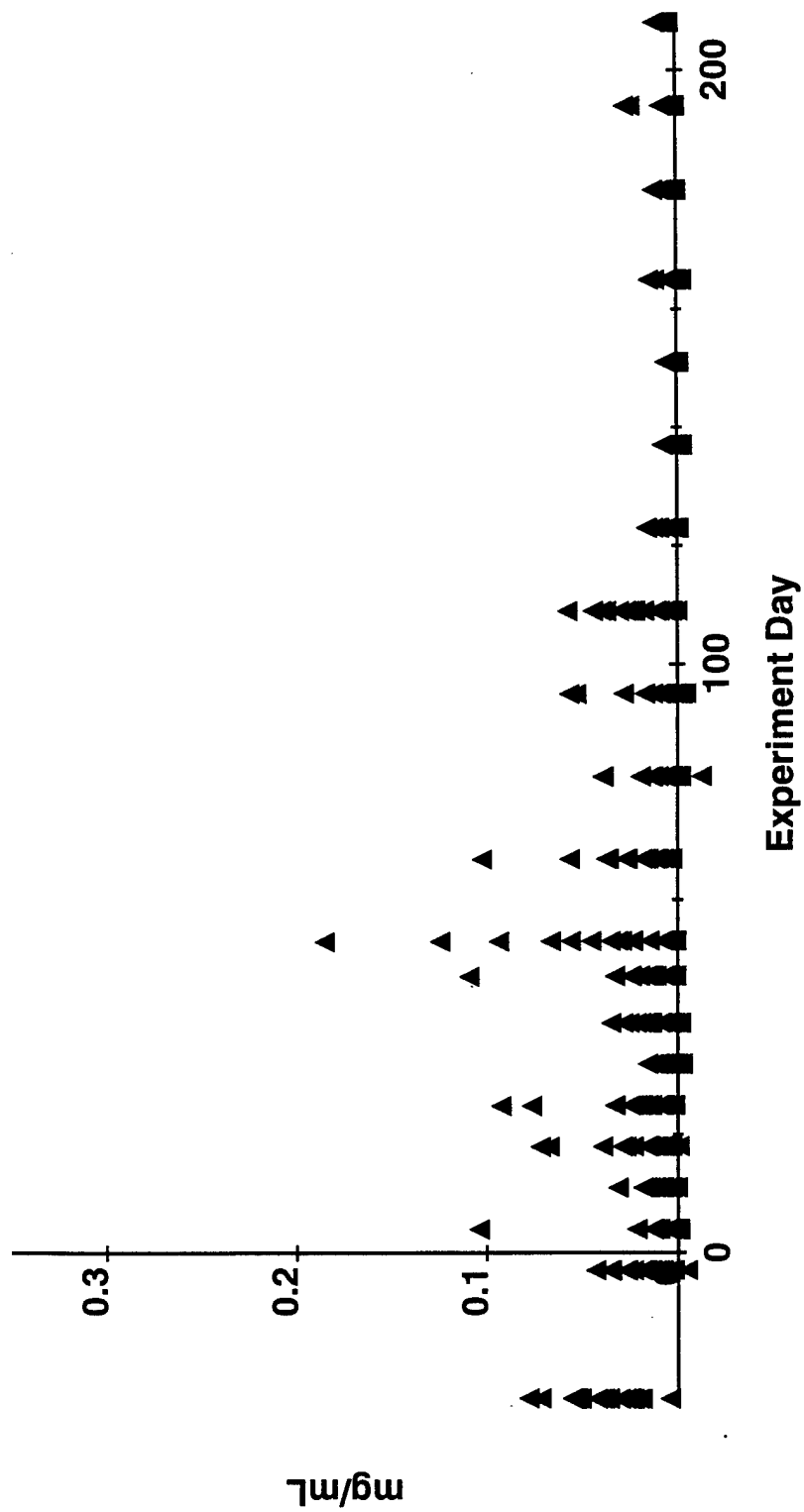
Figure 25a. Periphyton
Chlorophyll, High Dose
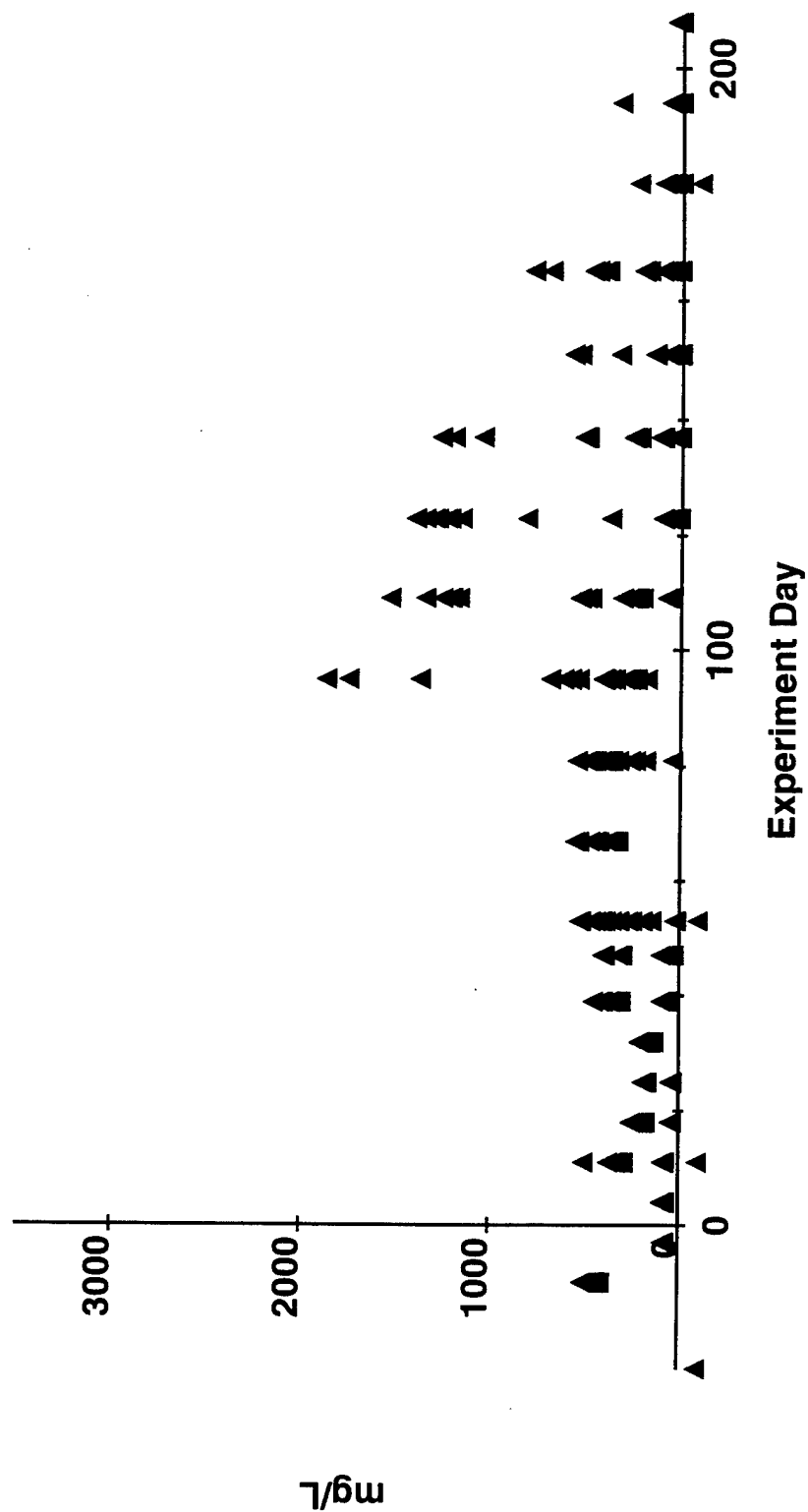
Figure 25b. Periphyton
Chlorophyll- Mid-Dose
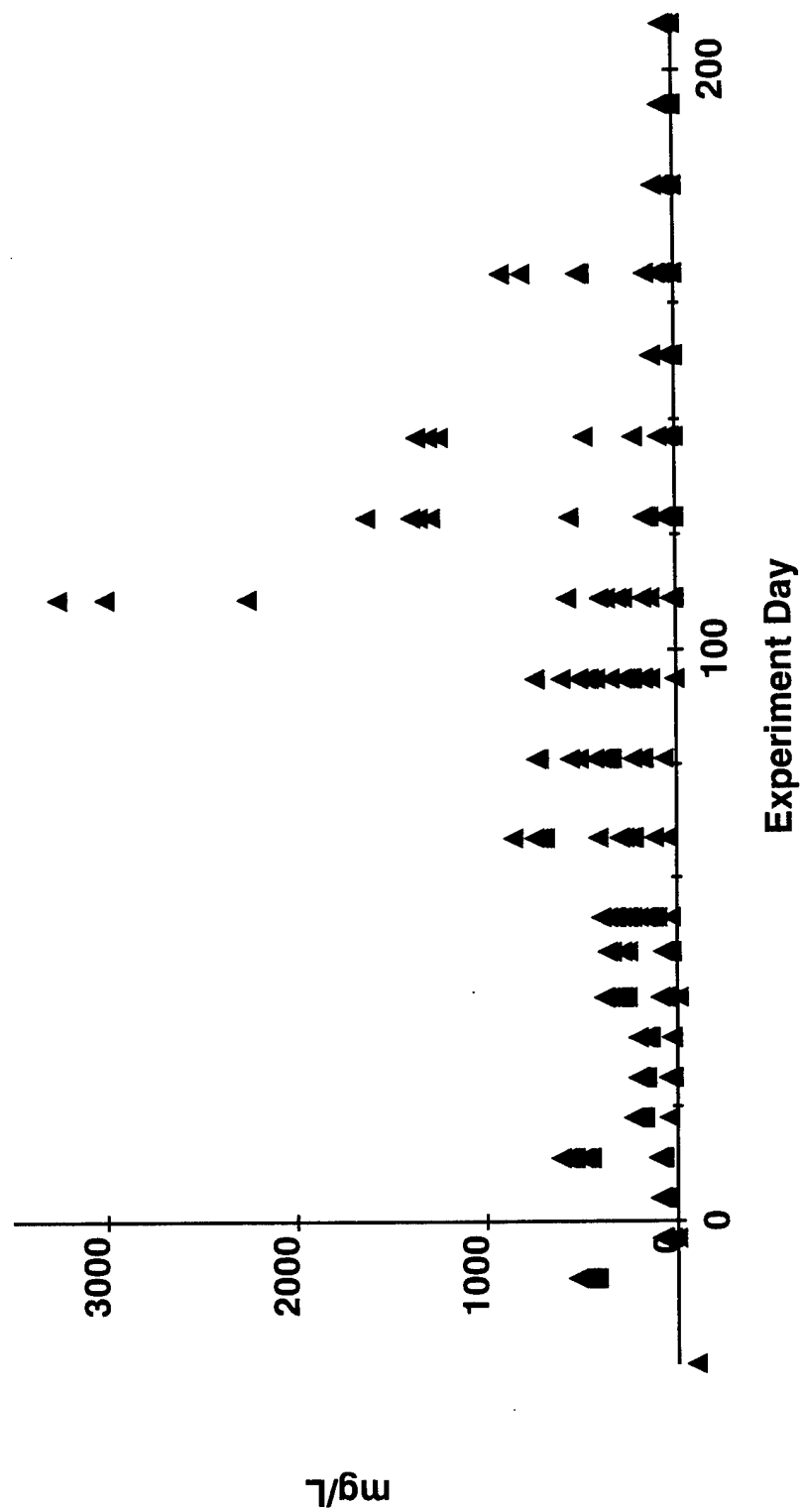
Figure 25c. Periphyton
Chlorophyll- Low Dose
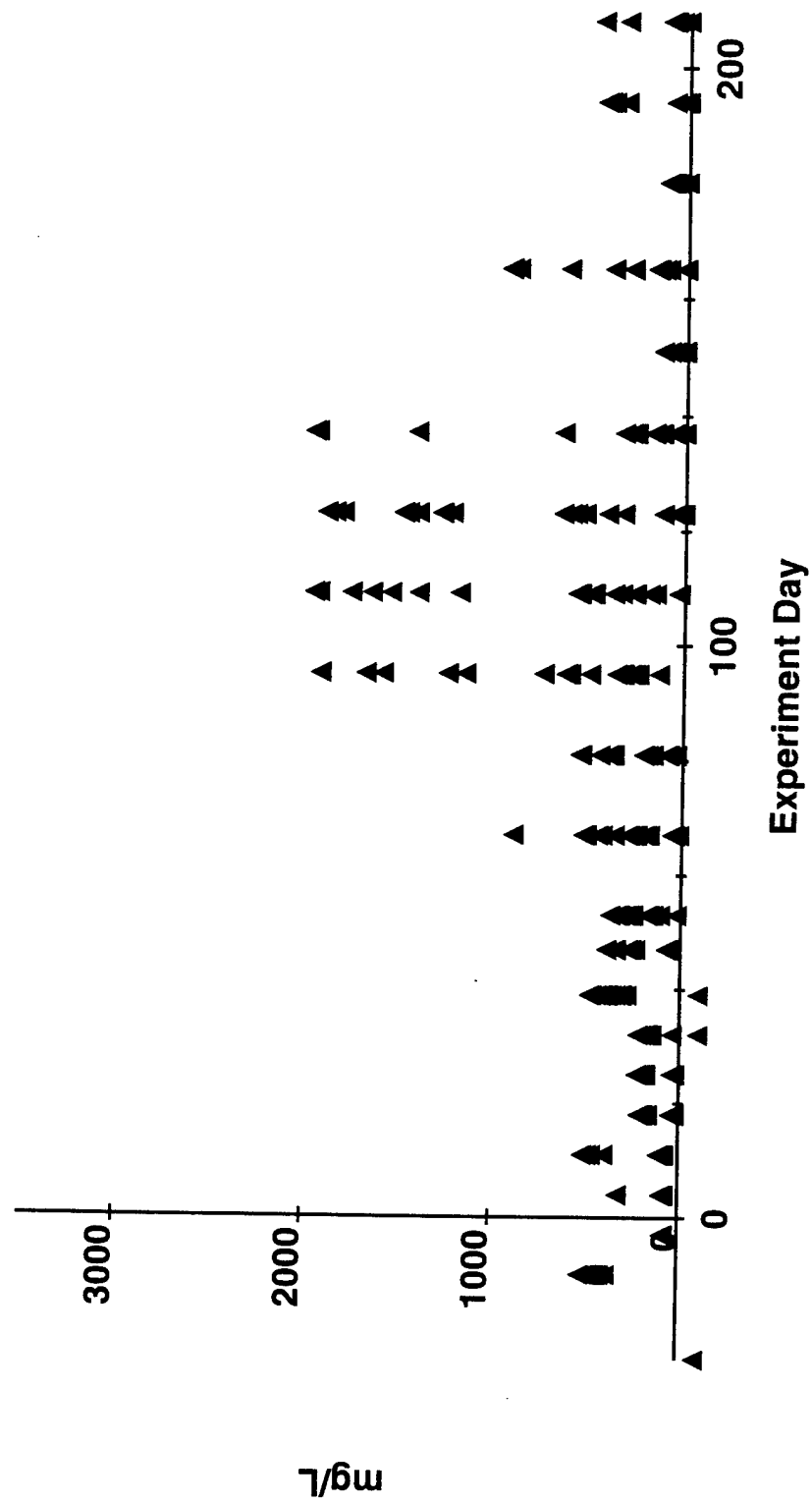
Figure 25d. Periphyton
Chlorophyll - Undosed
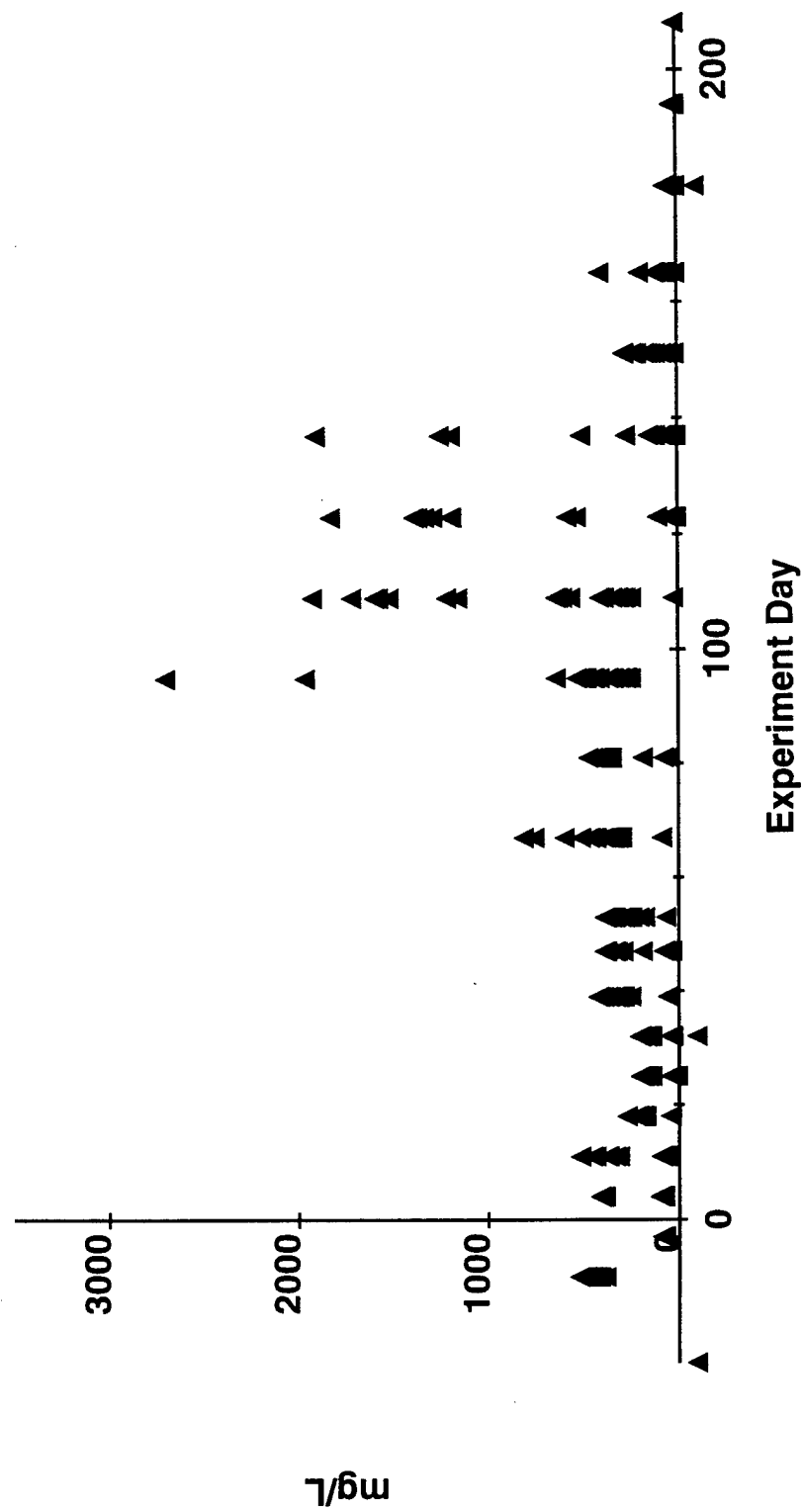
Figure 26a. Periphyton
Total Solids, High Dose
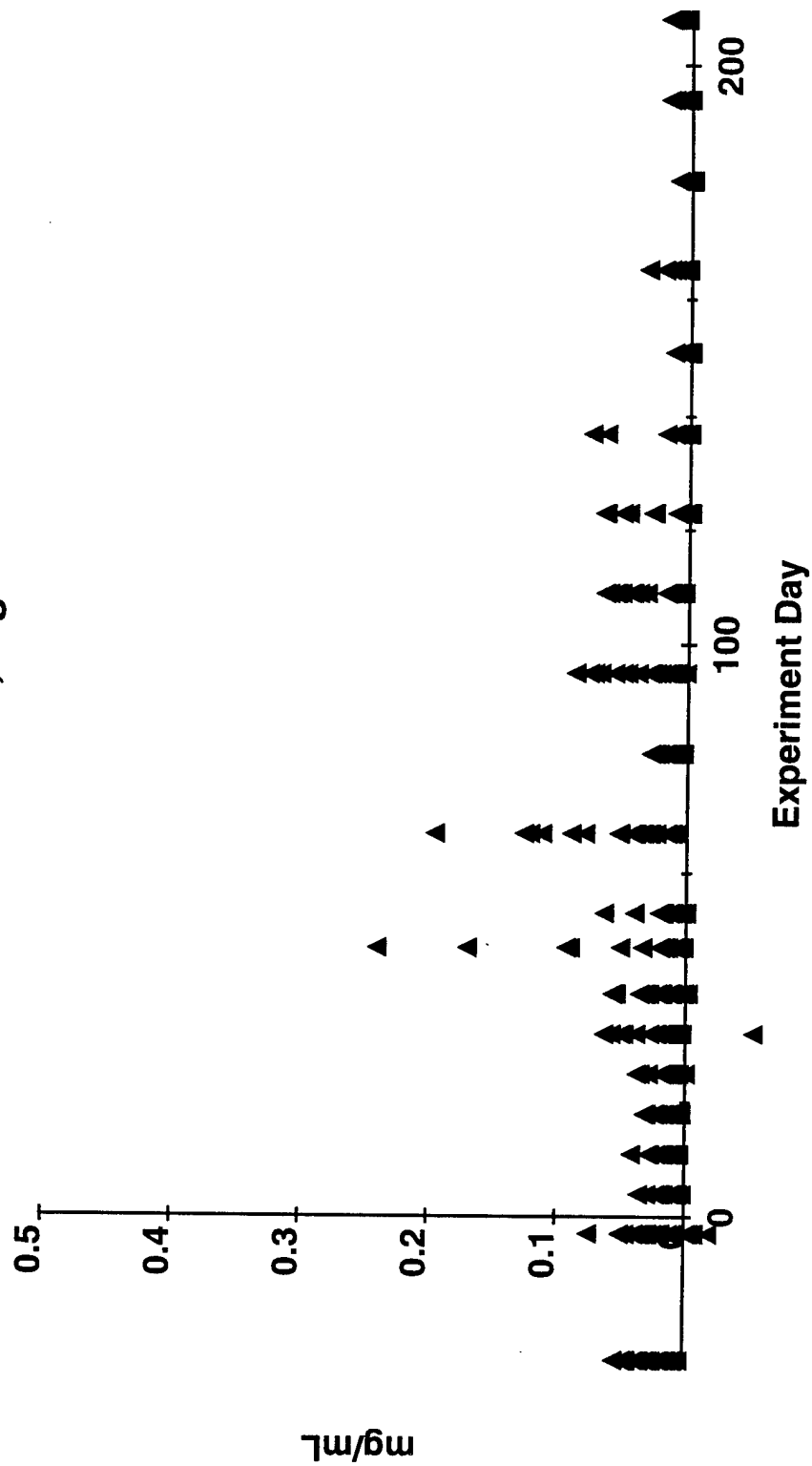
Figure 26b. Periphyton
Total Solids - Mid-Dose
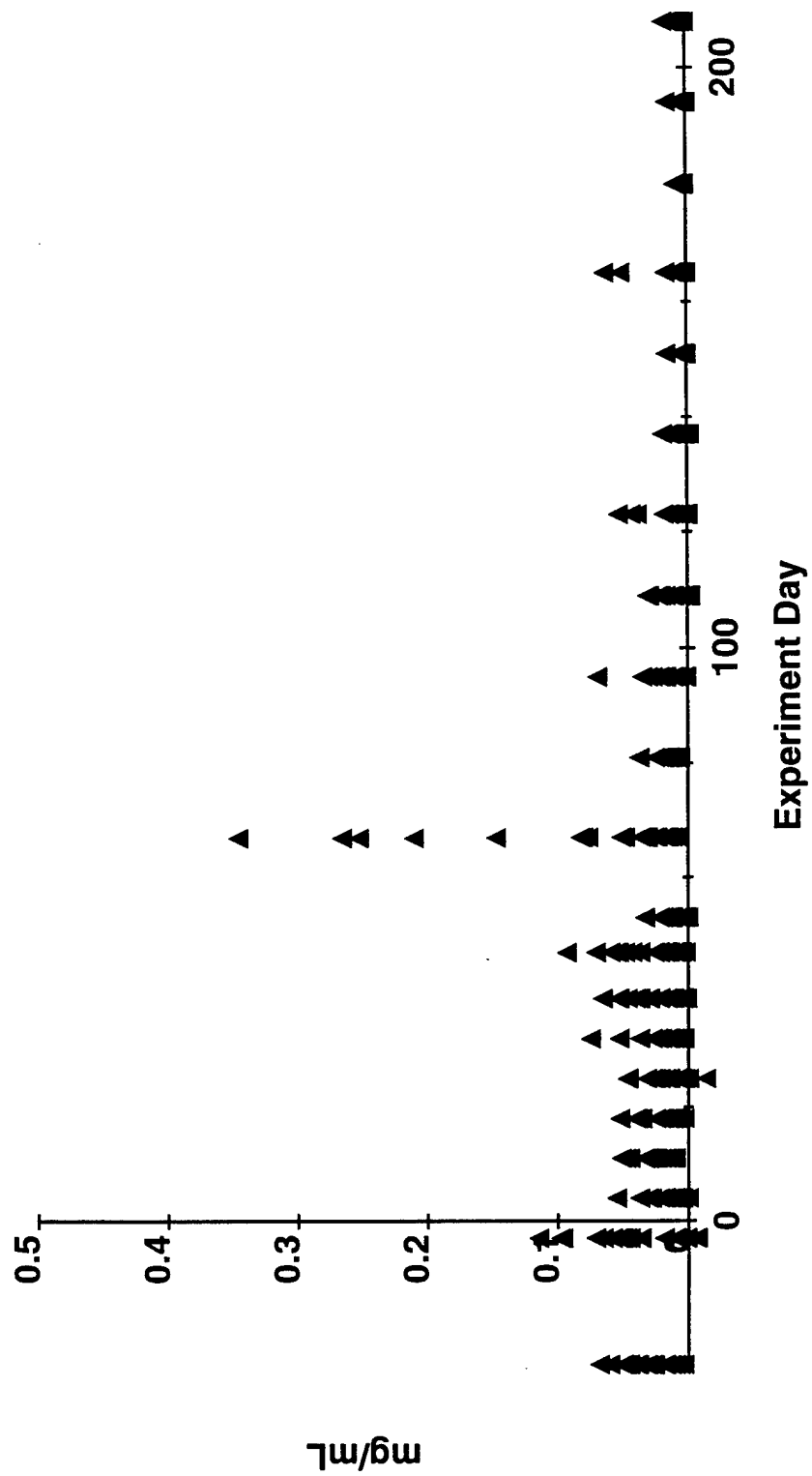
Figure 26c. Periphyton
Total Solids - Low Dose
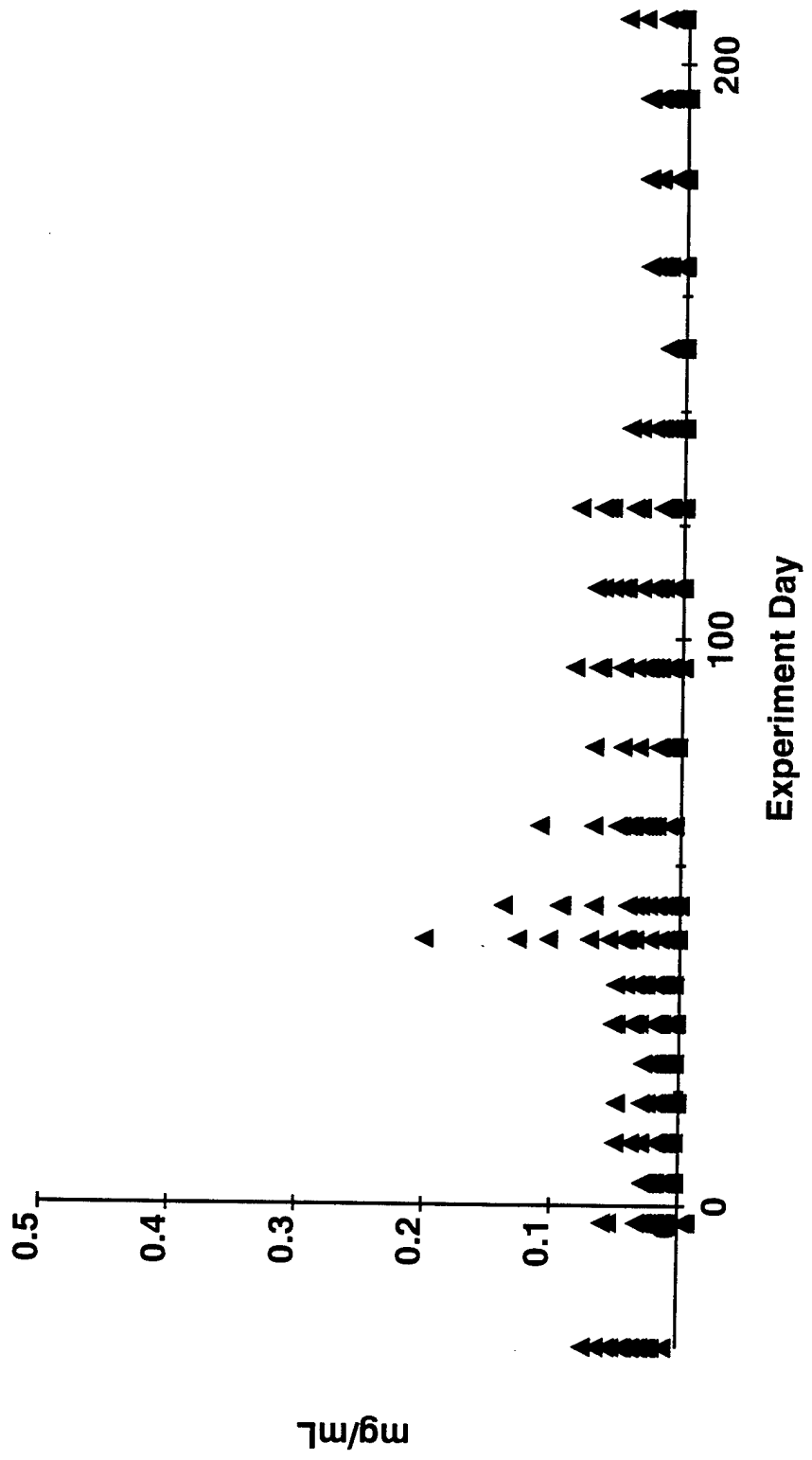
Figure 26d. Periphyton
Total Solids - Undosed

mg/mL

Experiment Day
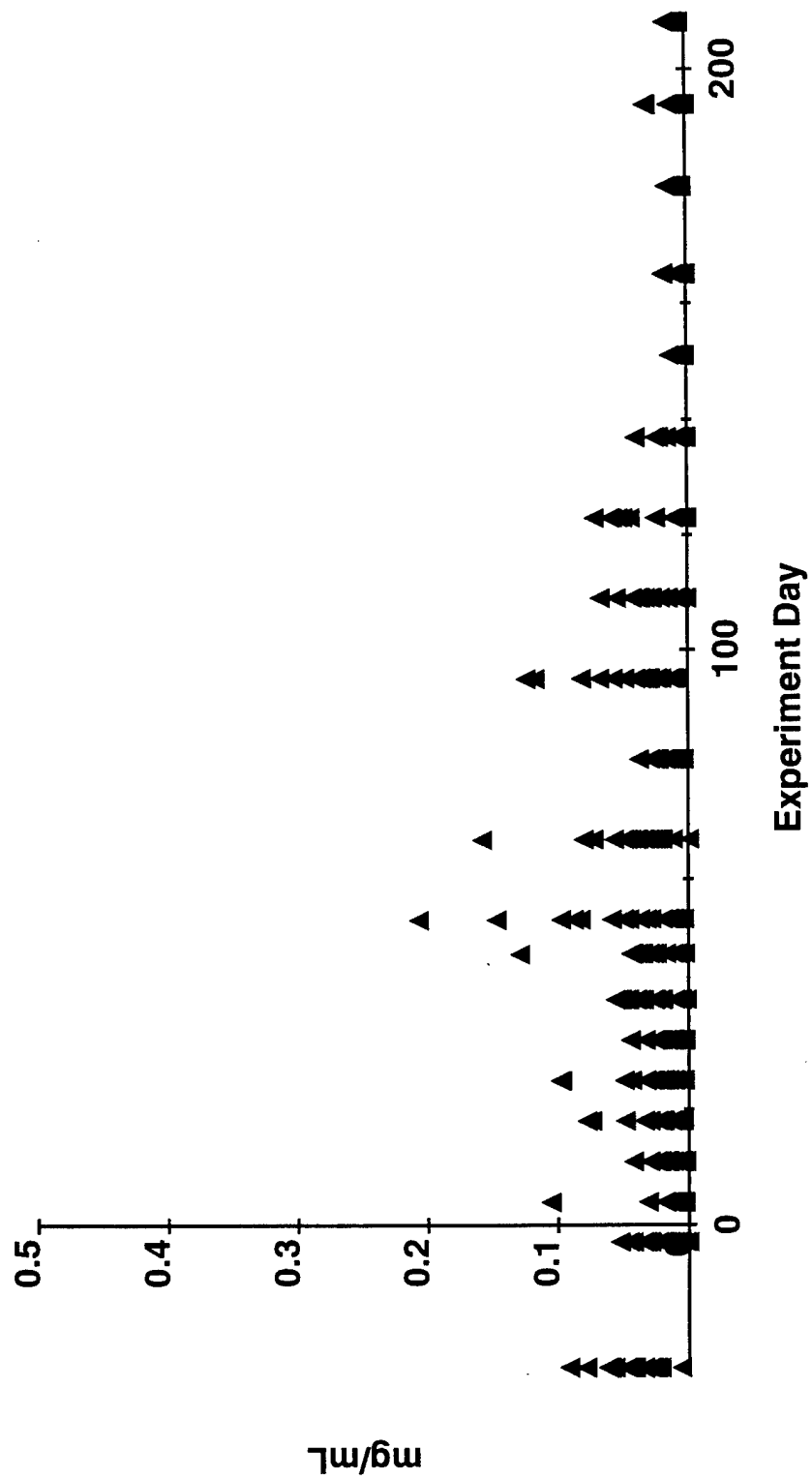
Figure 27a. Periphyton
Total Volatile Solids, High Dose
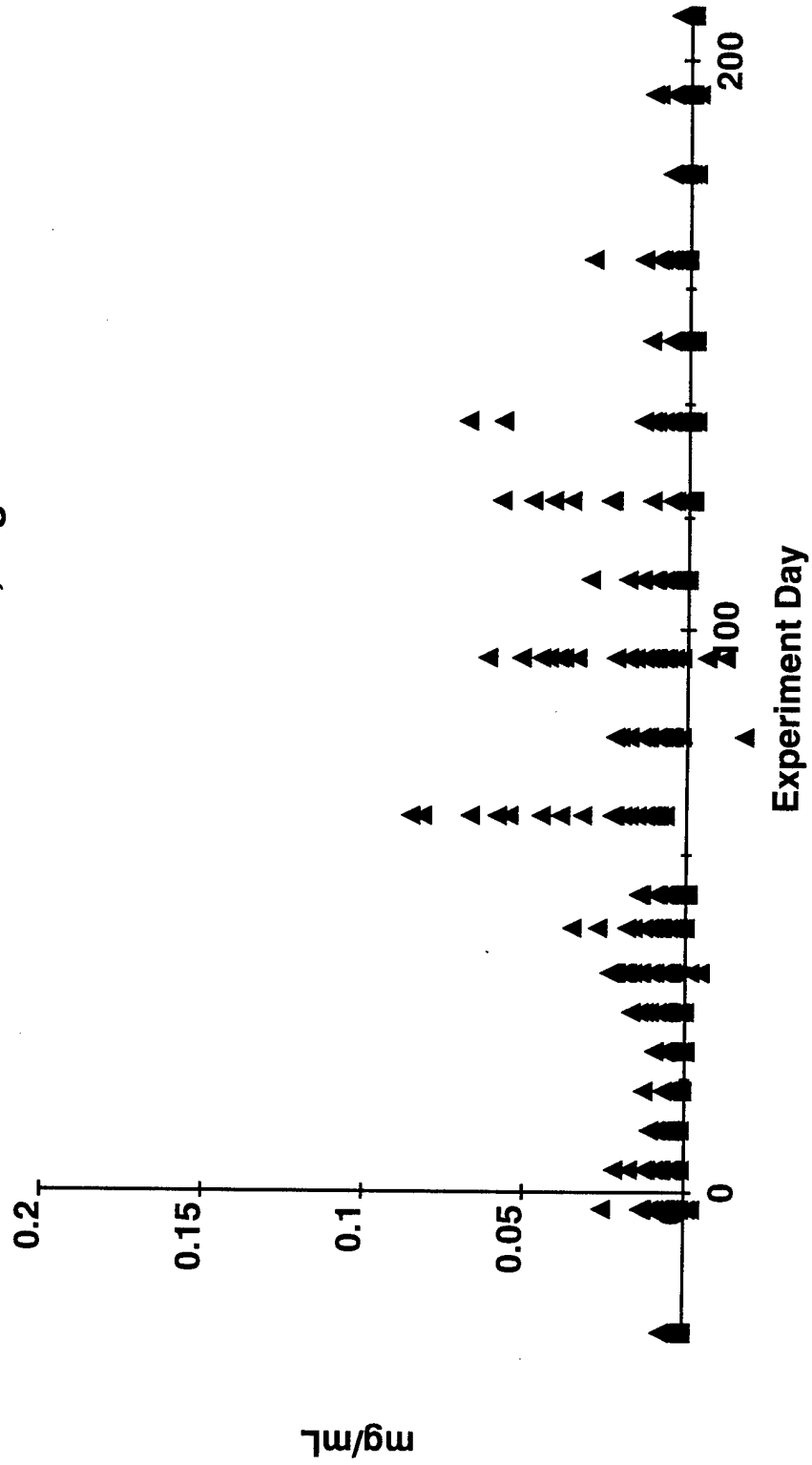
Figure 27b. Periphyton
Total Volatile Solids - Mid-Dose
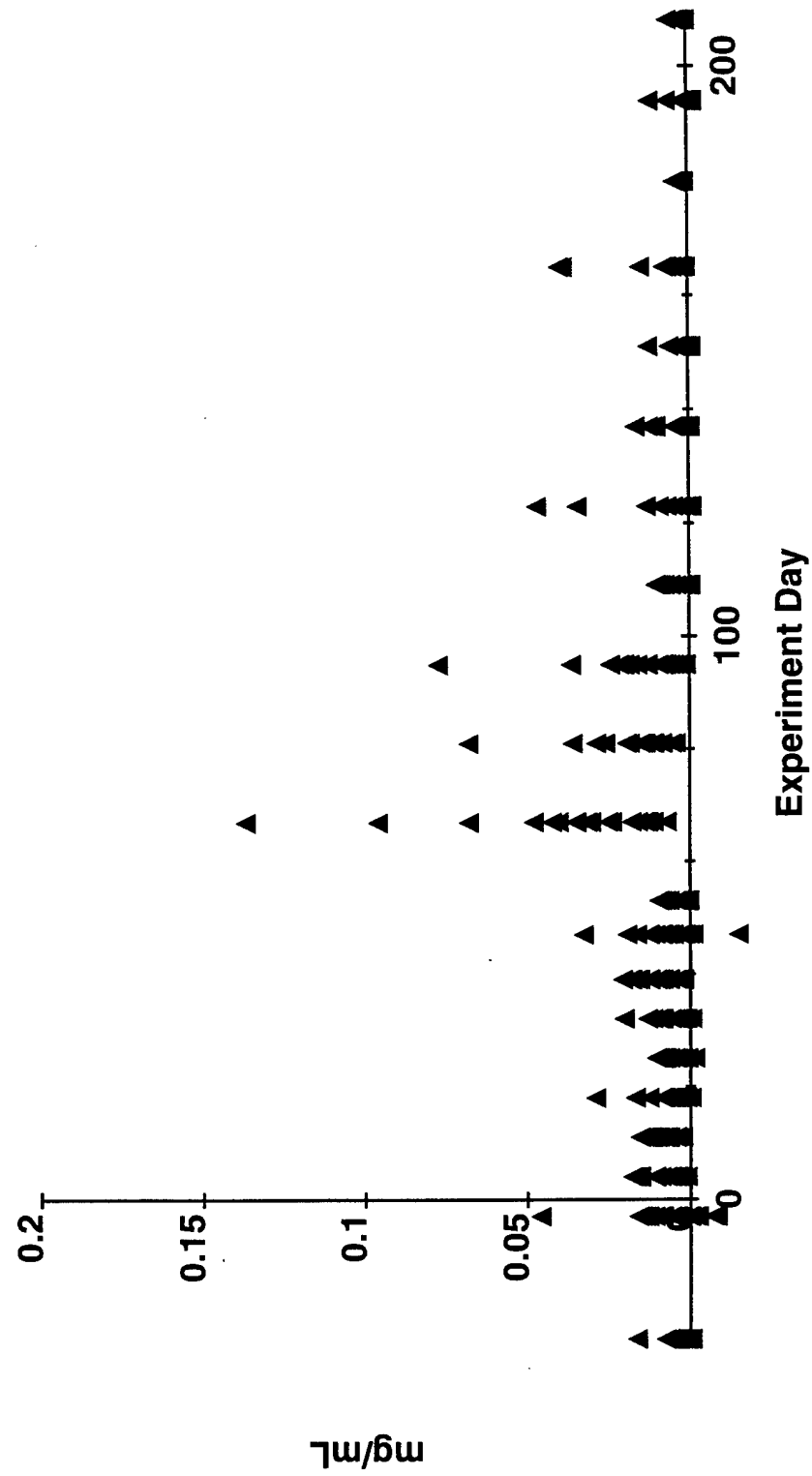
Figure 27c. Periphyton
Total Solids - Low Dose

Experiment Day

mg/mL
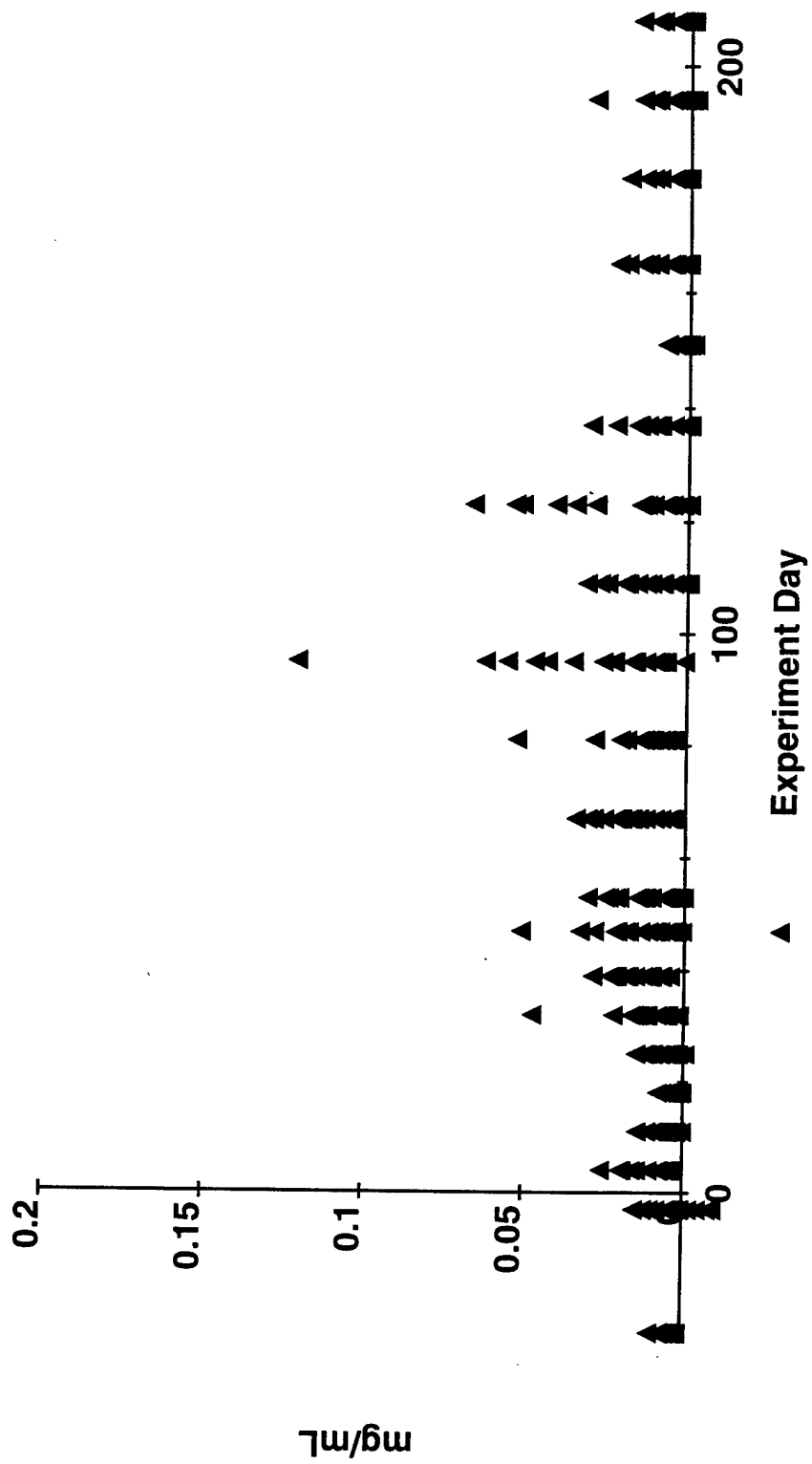
Figure 27d. Periphyton
Total Volatile Solids - Undosed
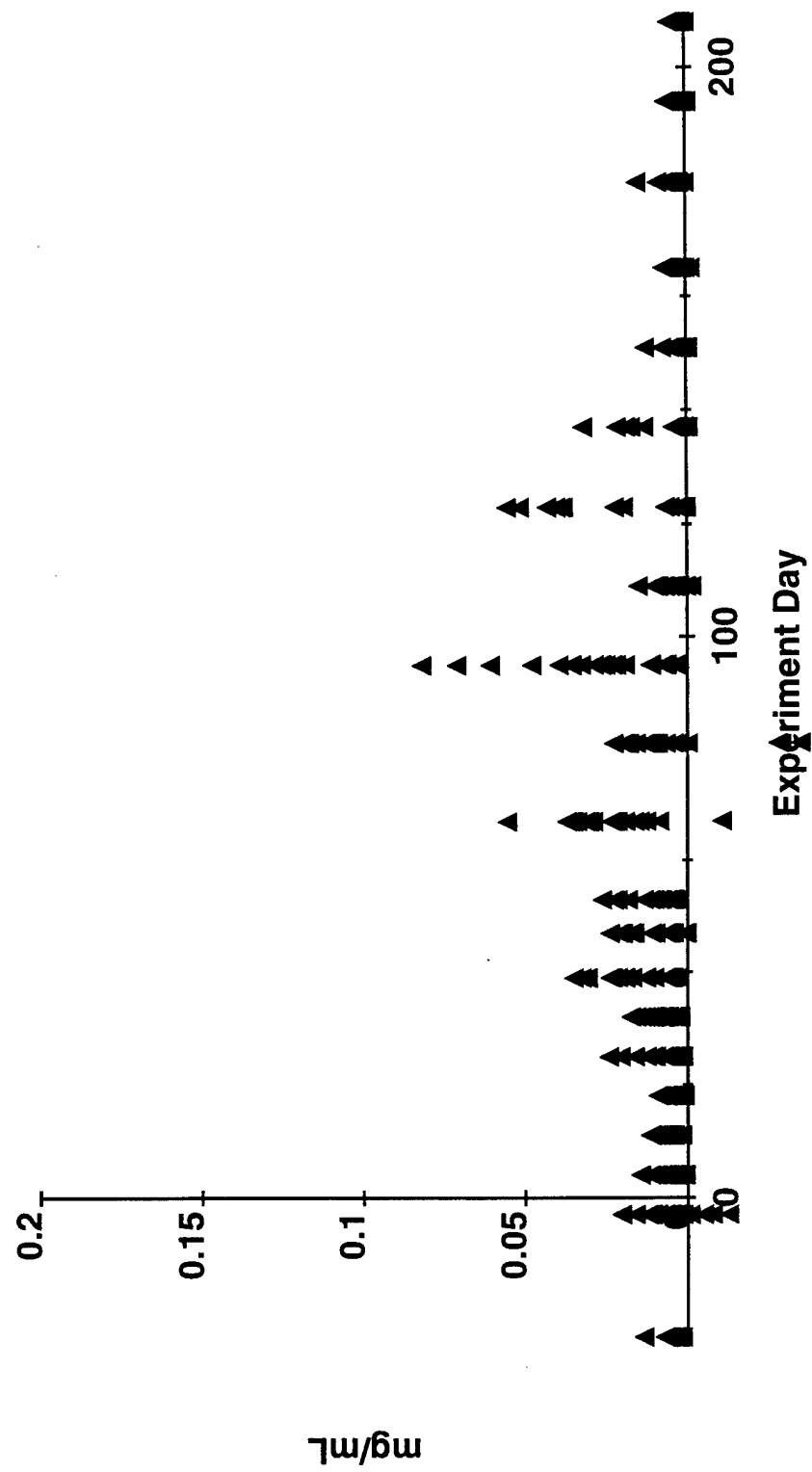
Figure 28a. Periphyton
TTC Respiration, High Dose
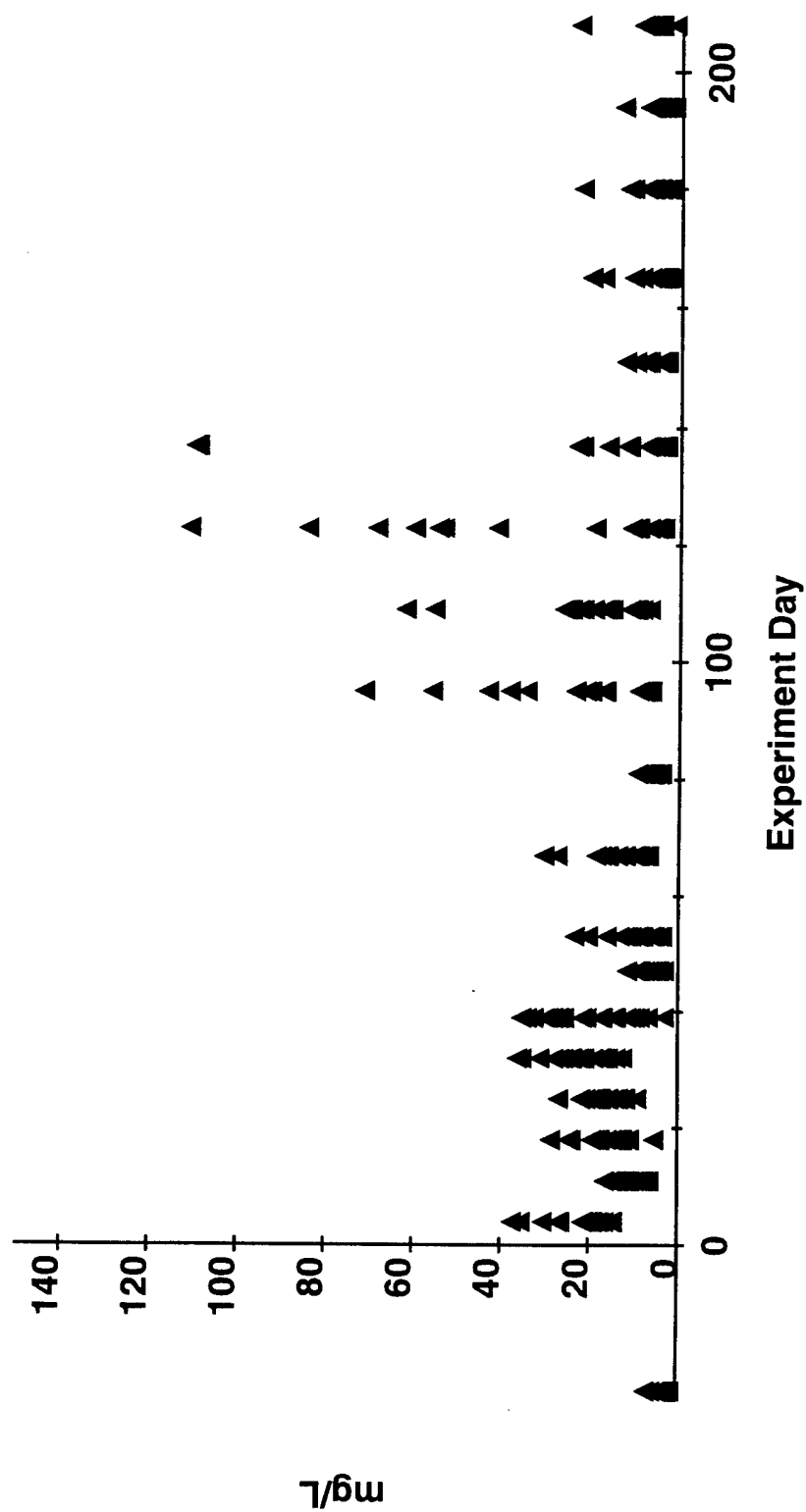
Figure 28b. Periphyton
TTC Respiration- Mid-Dose
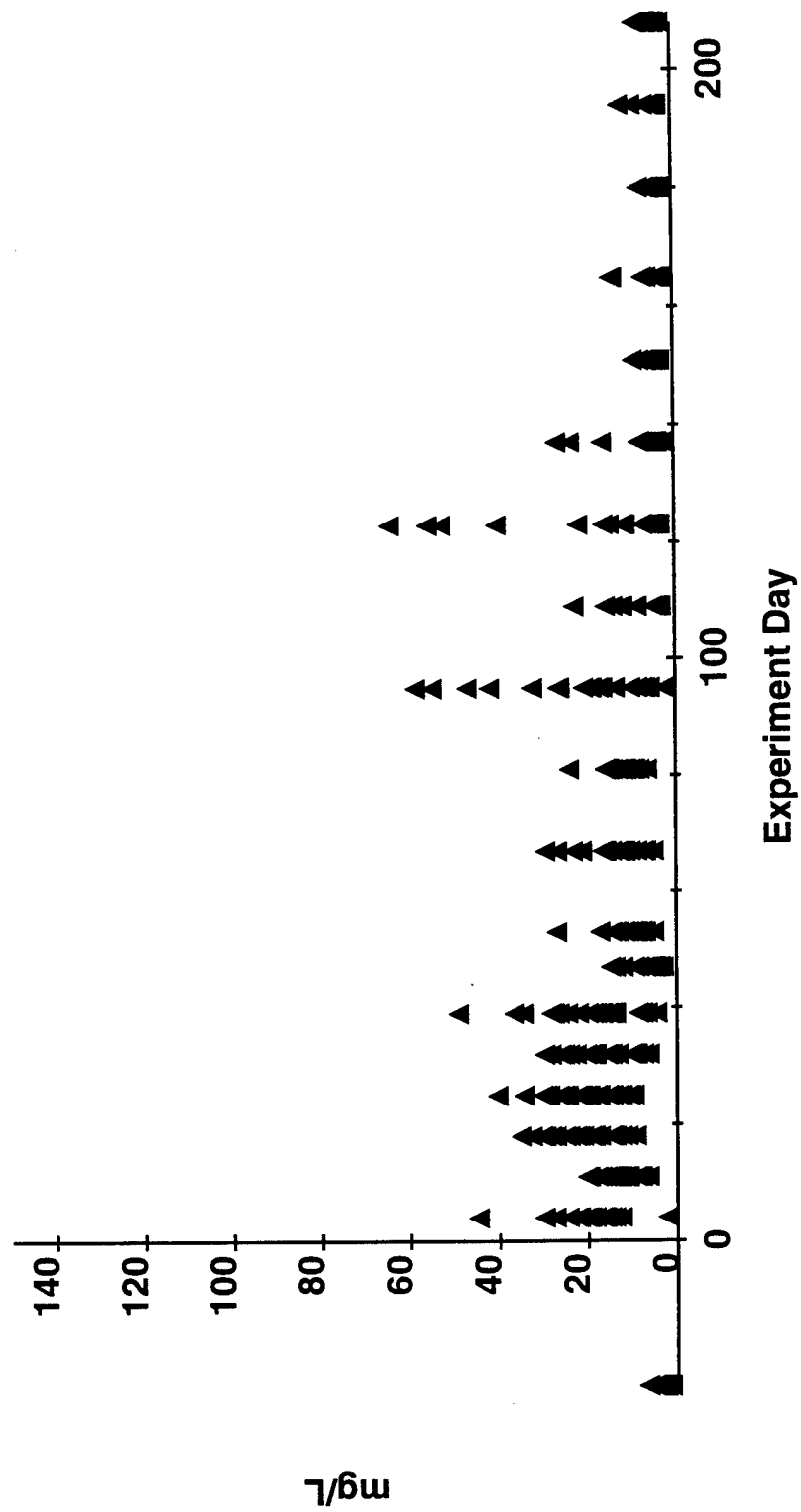
Figure 28c. Periphyton
TTC Respiration- Low Dose
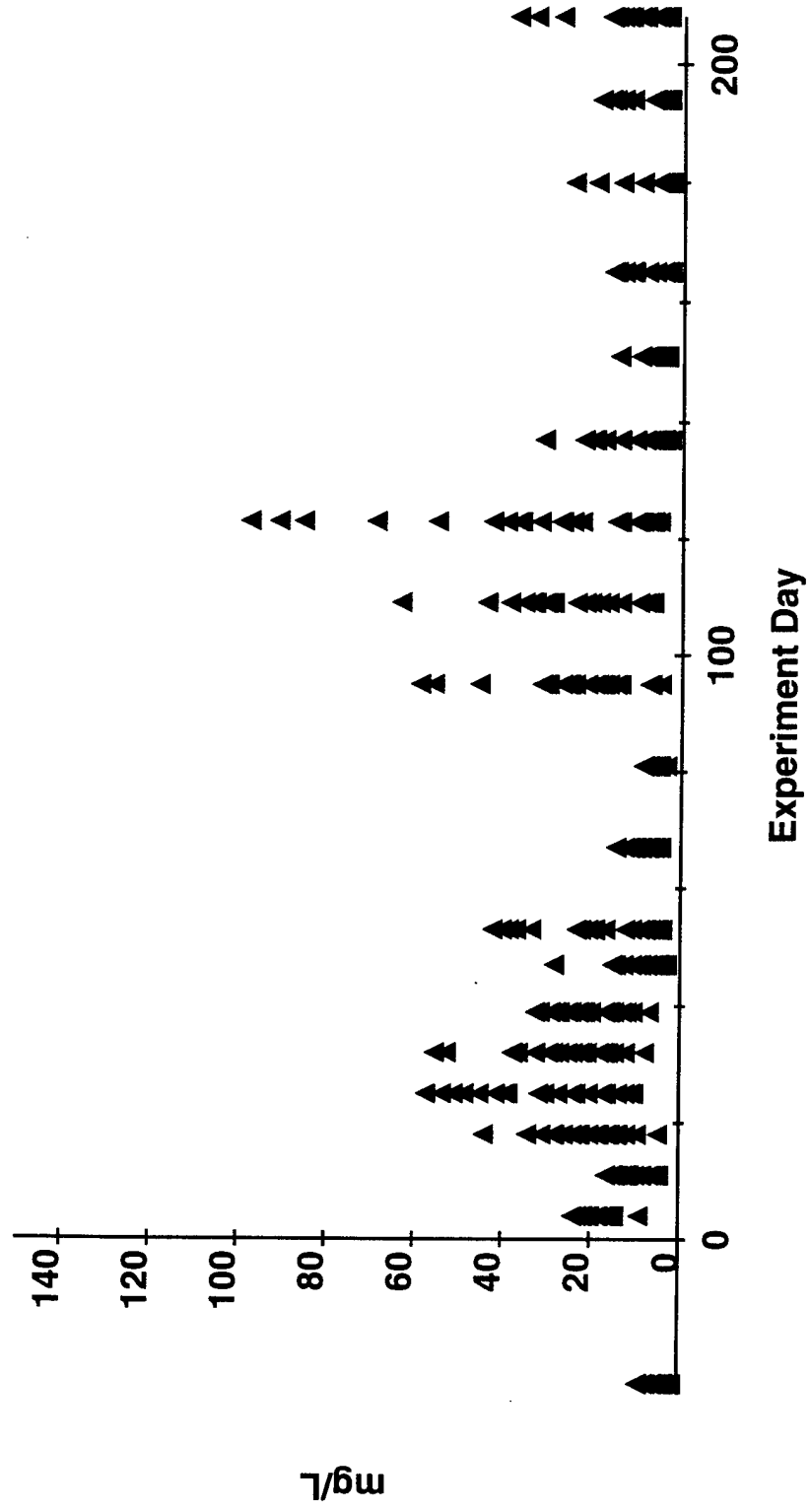
Figure 28d. Periphyton
TTC Respiration - Undosed
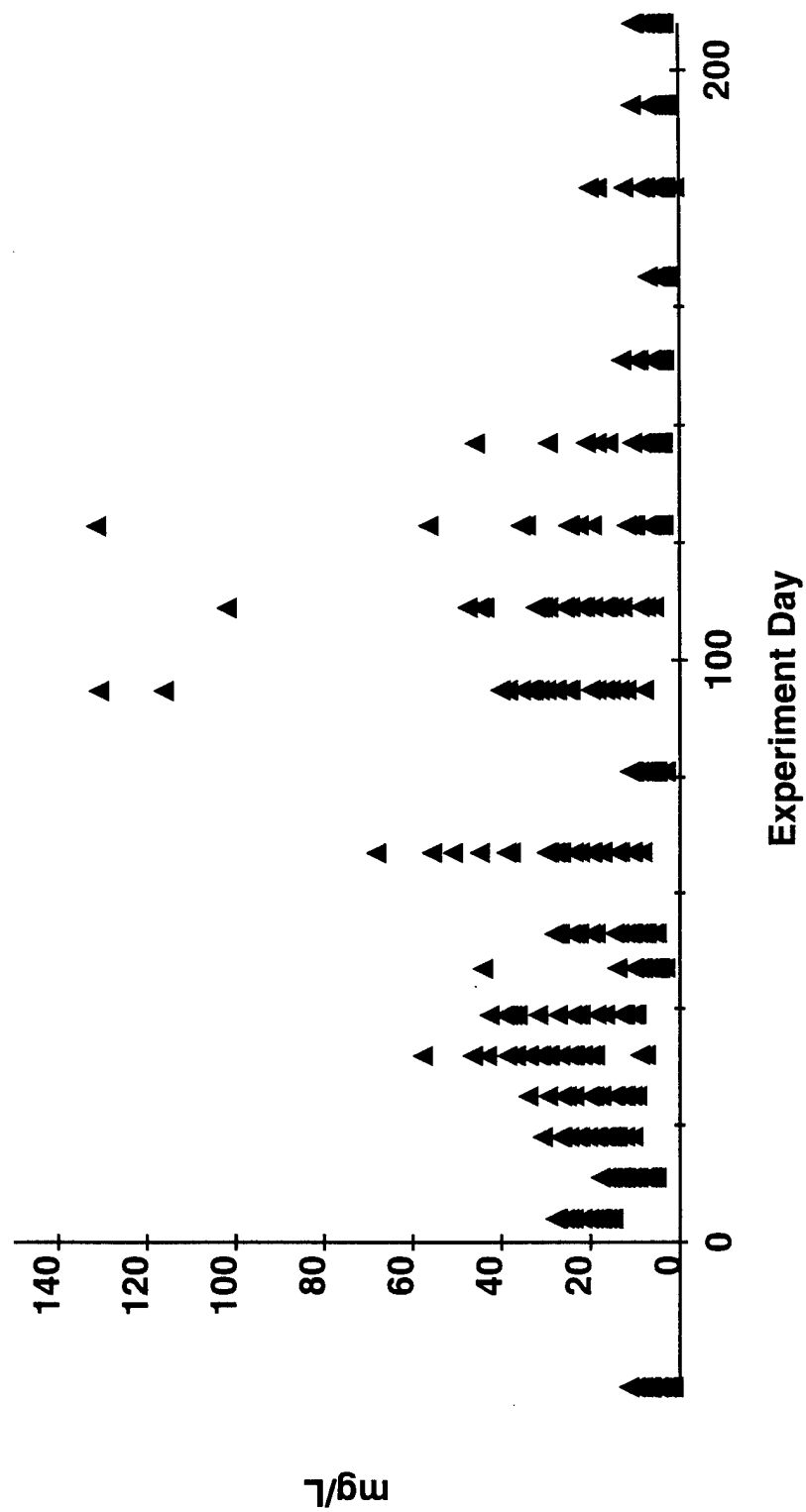
Figure 29a. Annelids
Bloodworms - High Dose
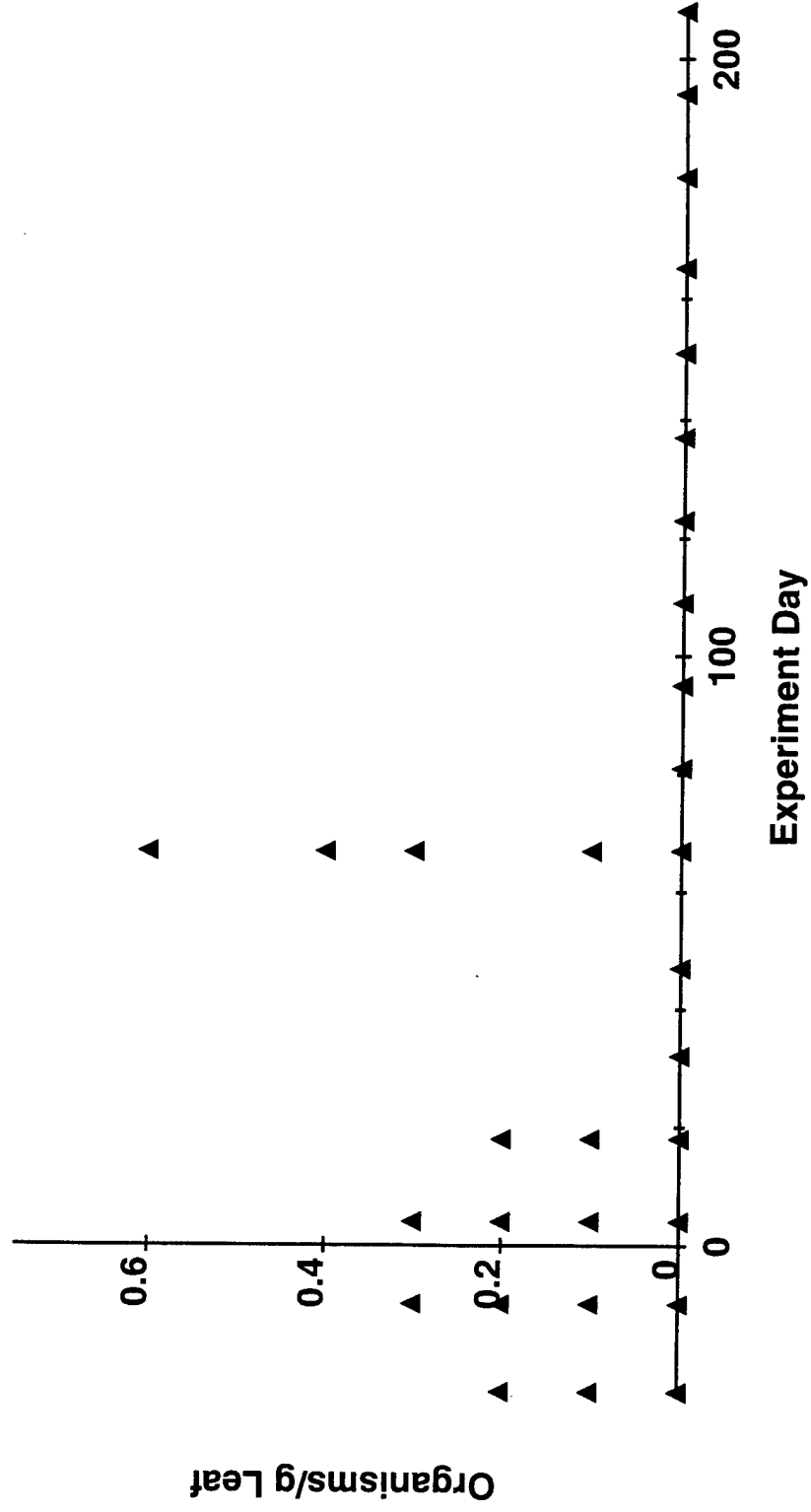
Figure 29b. Annelids
Bloodworms - Mid-Dose
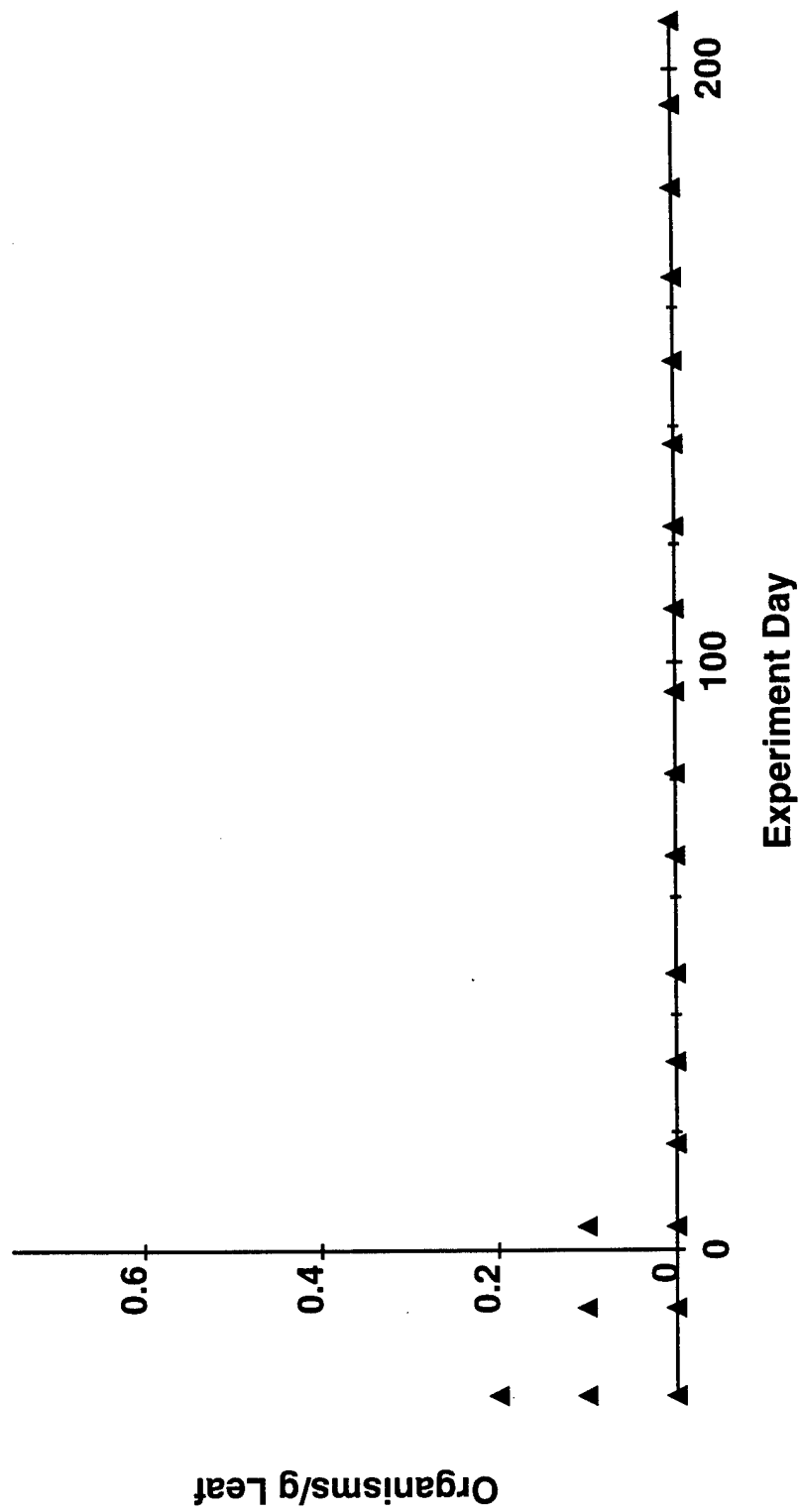
Figure 29c. Annelids
Bloodworms - Low Dose

Organisms/g Leaf

Experiment Day
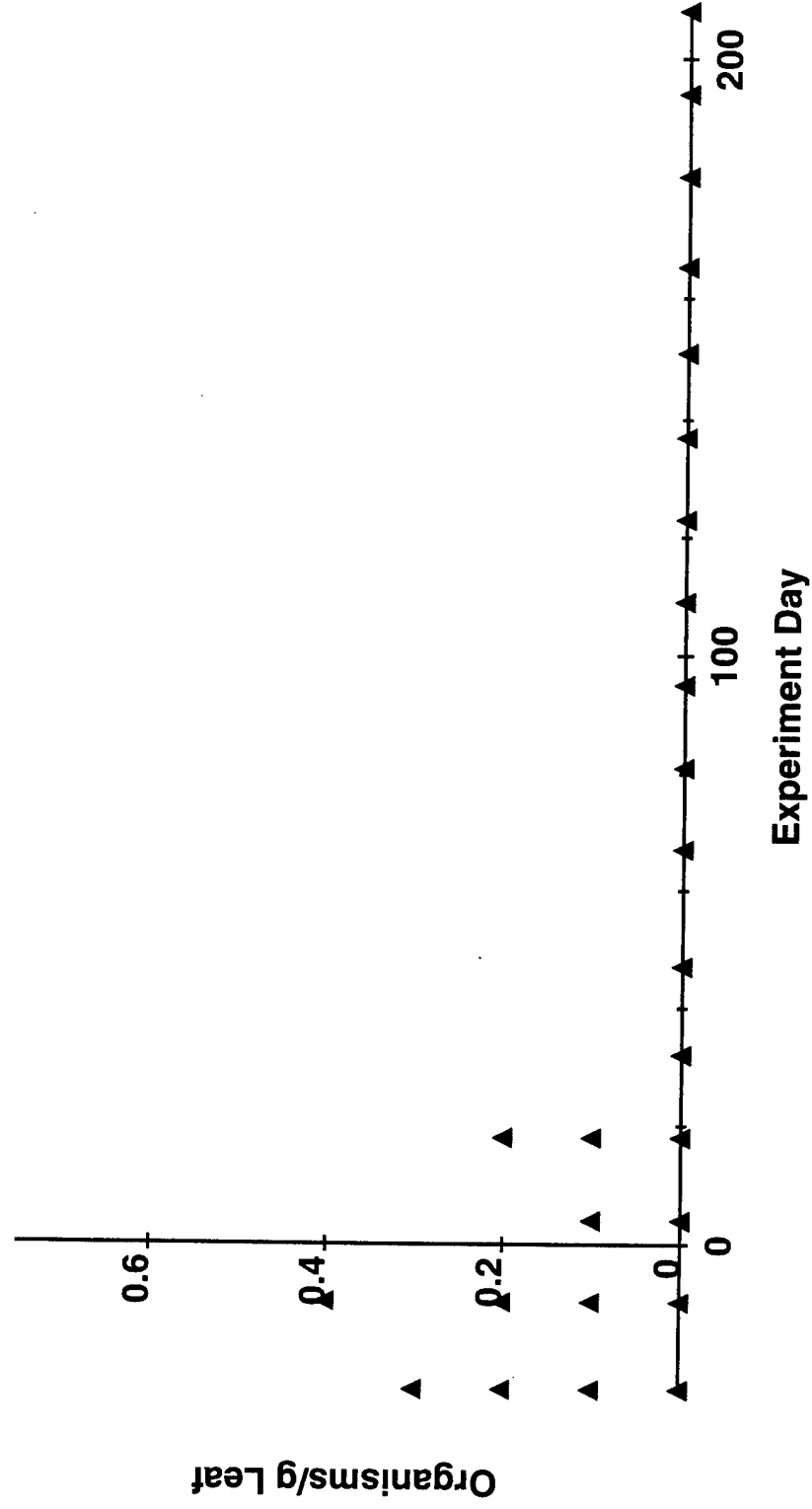
Figure 29d. Annelids
Bloodworms - Undosed
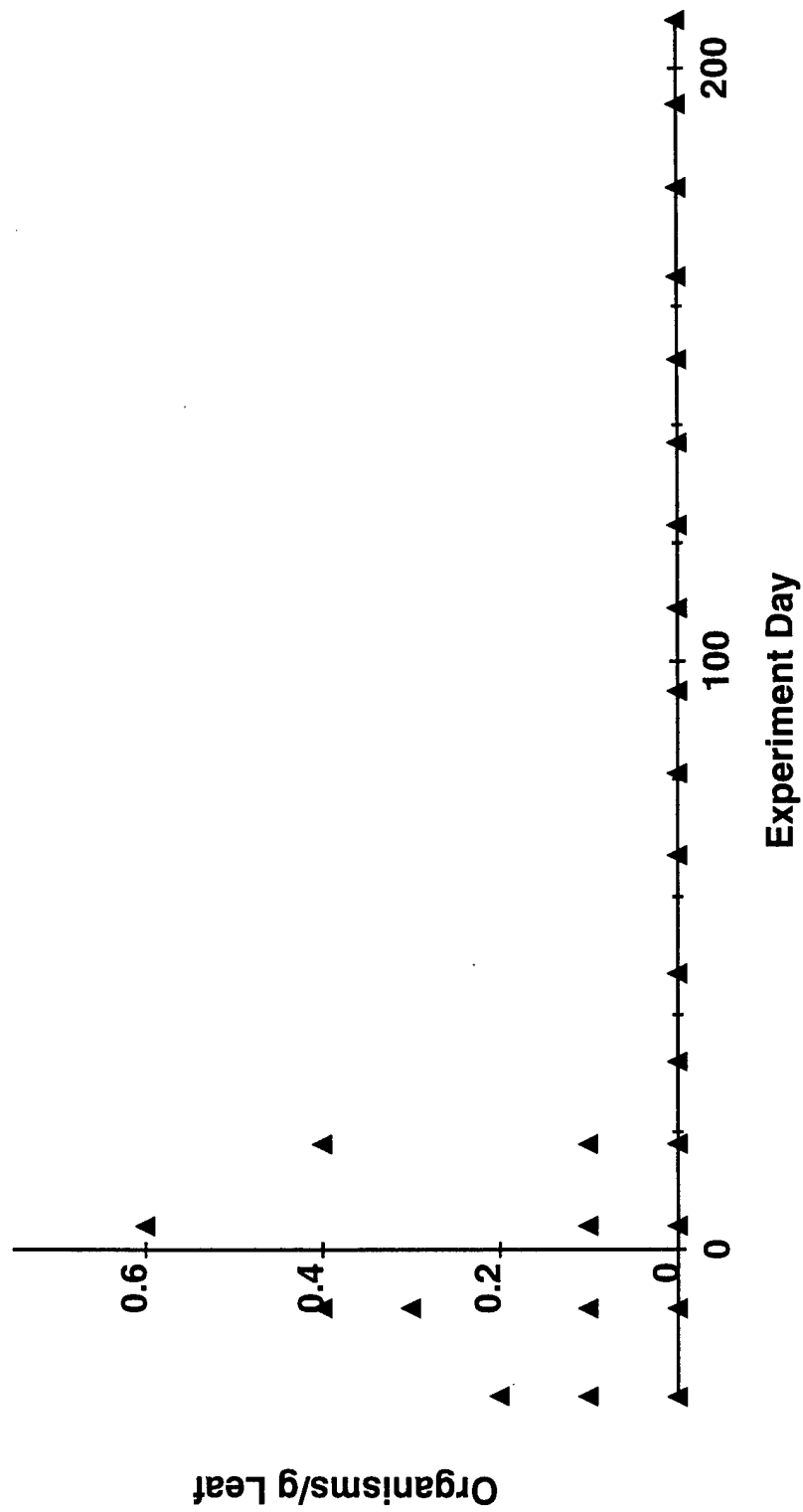
Figure 30a. Annelids
Other - High Dose
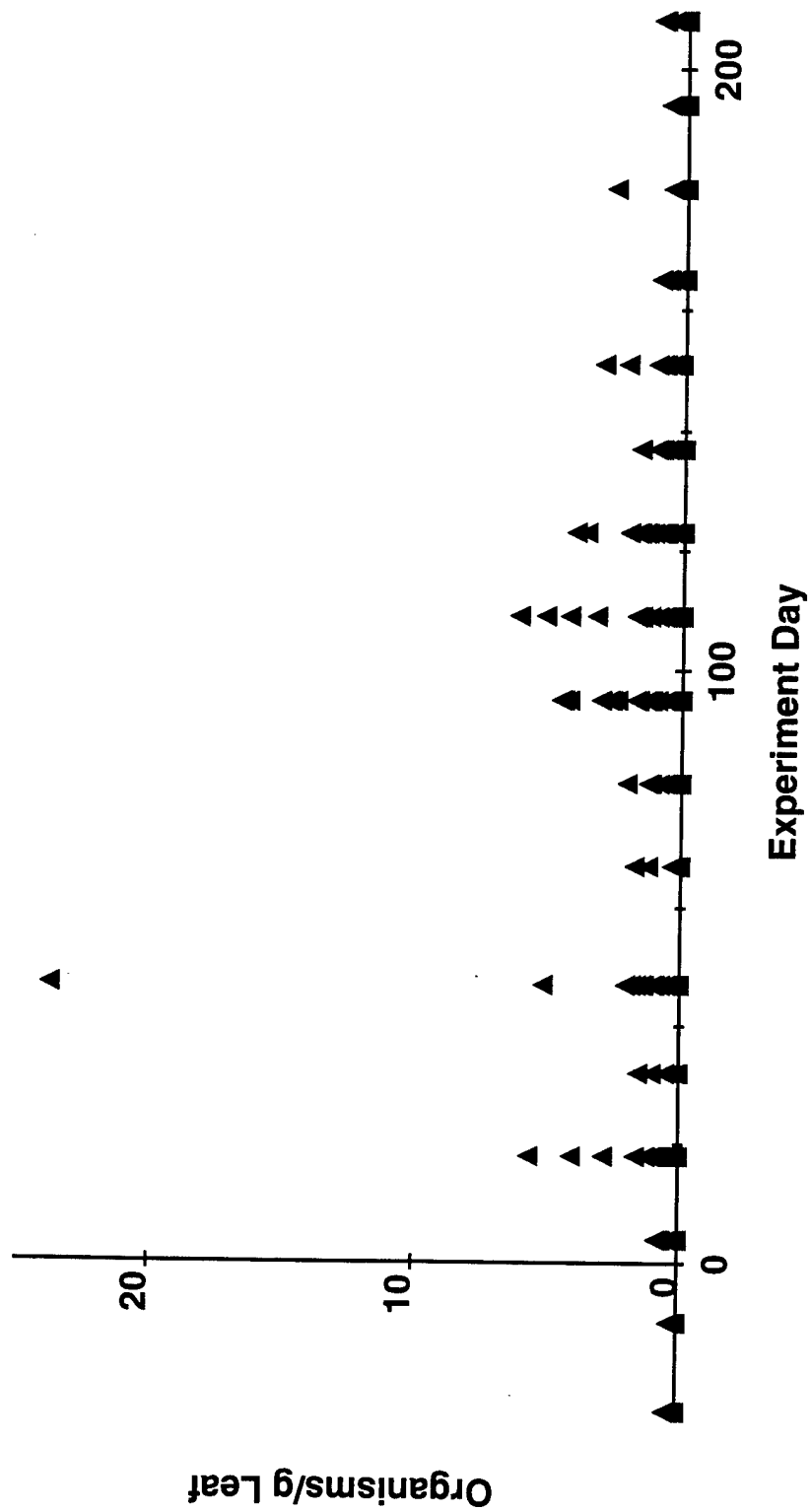
Figure 30b. Annelids
Bloodworms - Mid-Dose
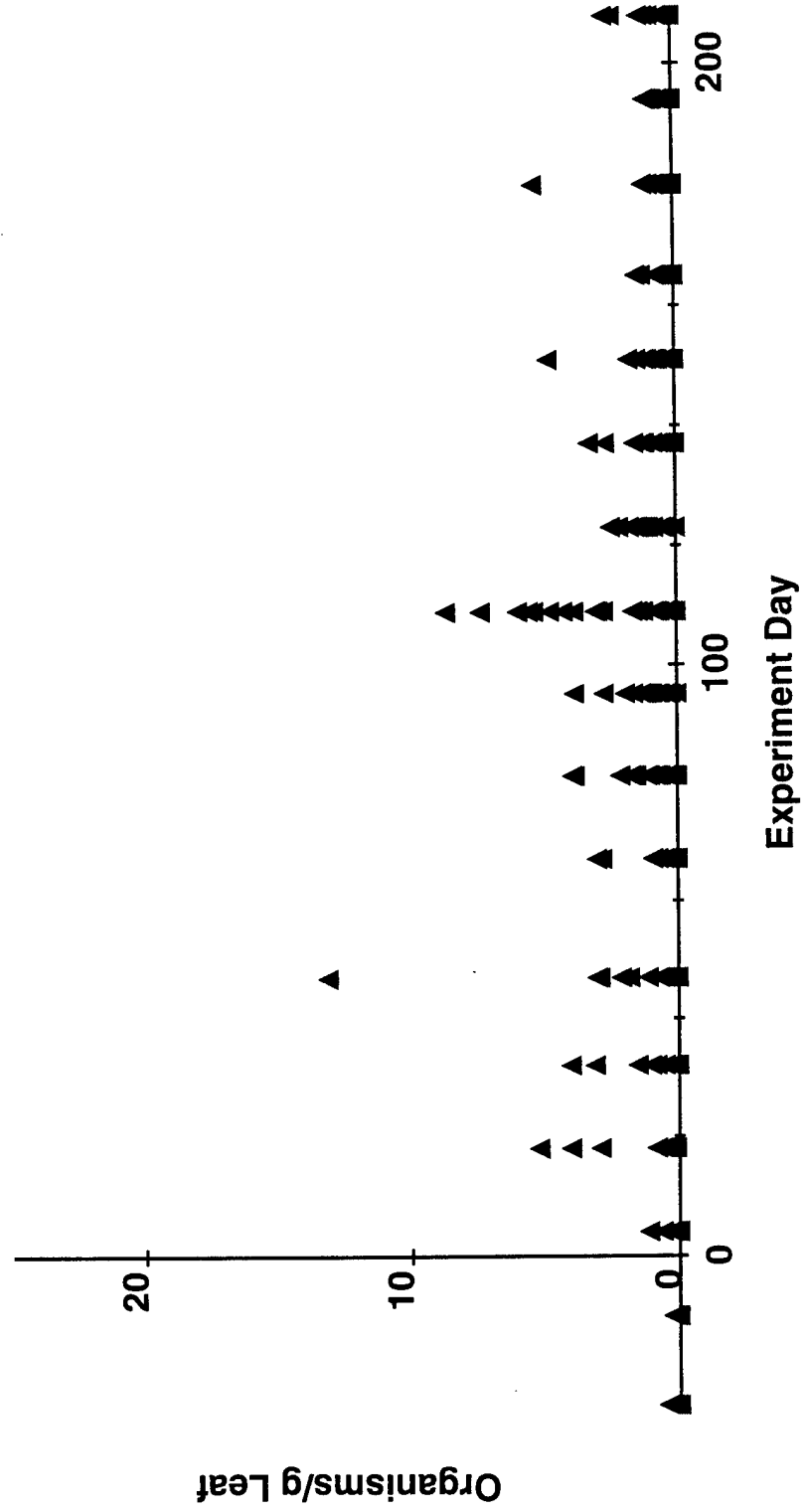
Figure 30c. Annelids
Other - Low Dose
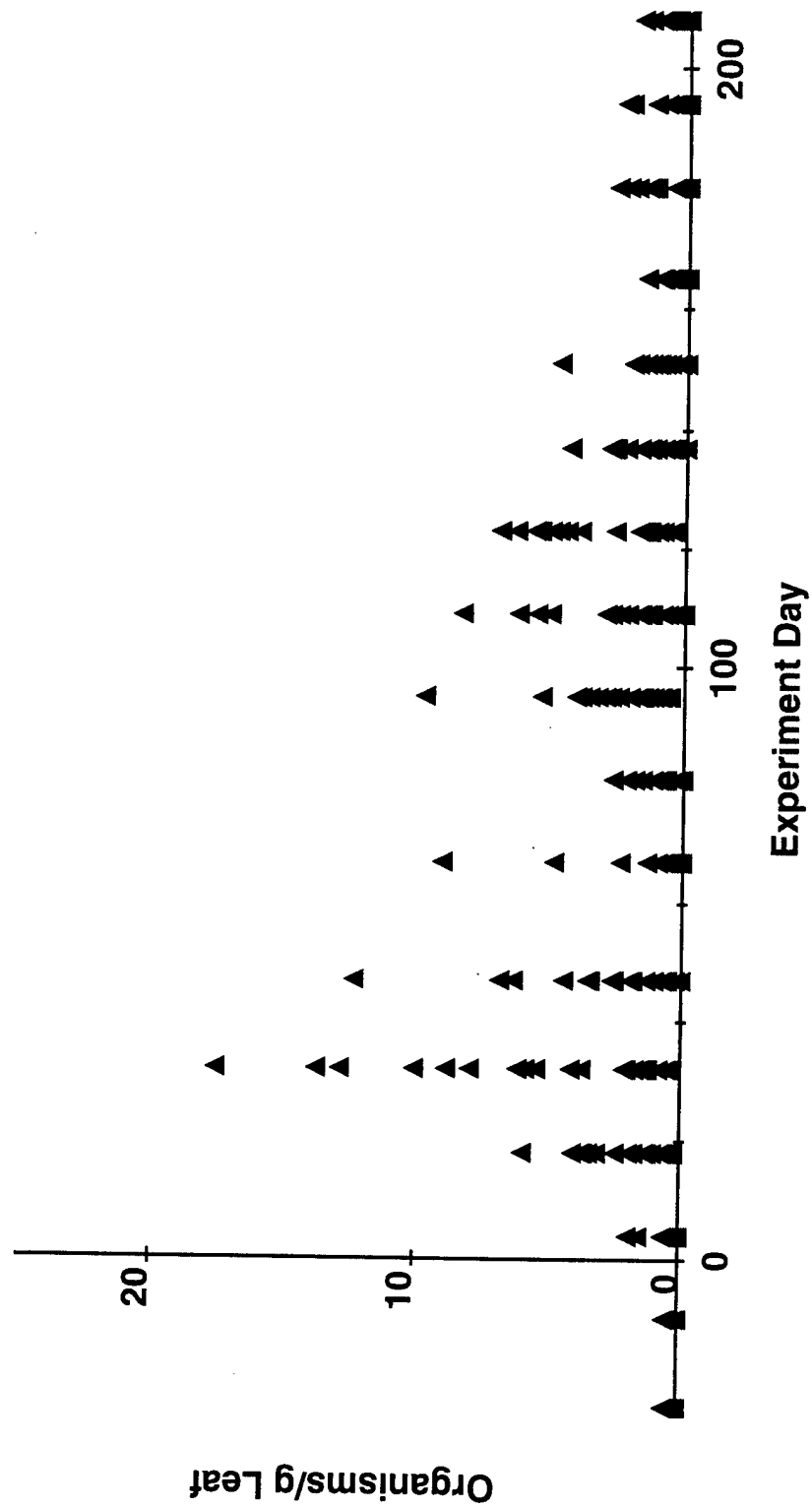
Figure 30d. Annelids
Other - Undosed
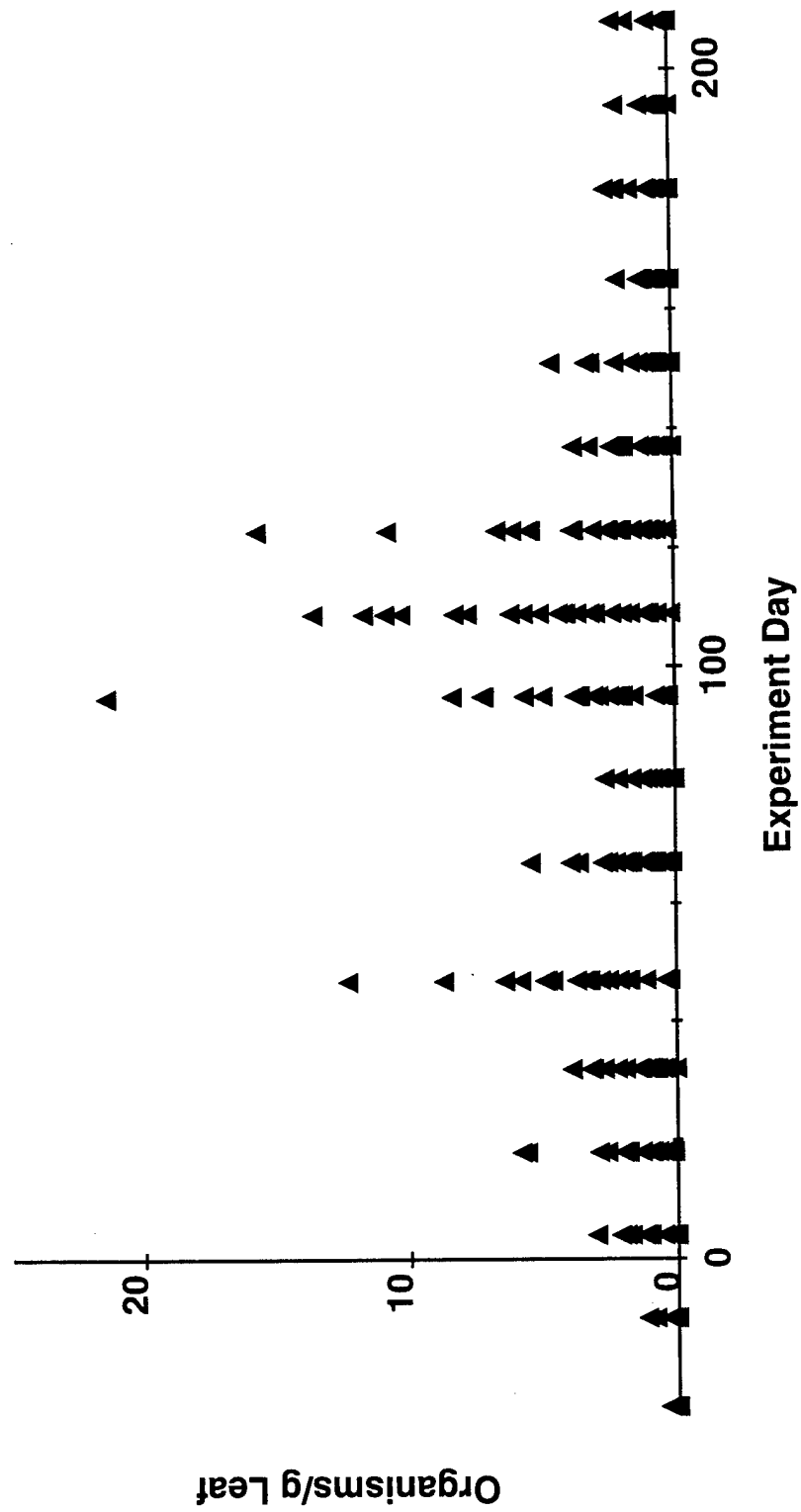
Figure 31a. Crustaceae
Amphipods - High Dose
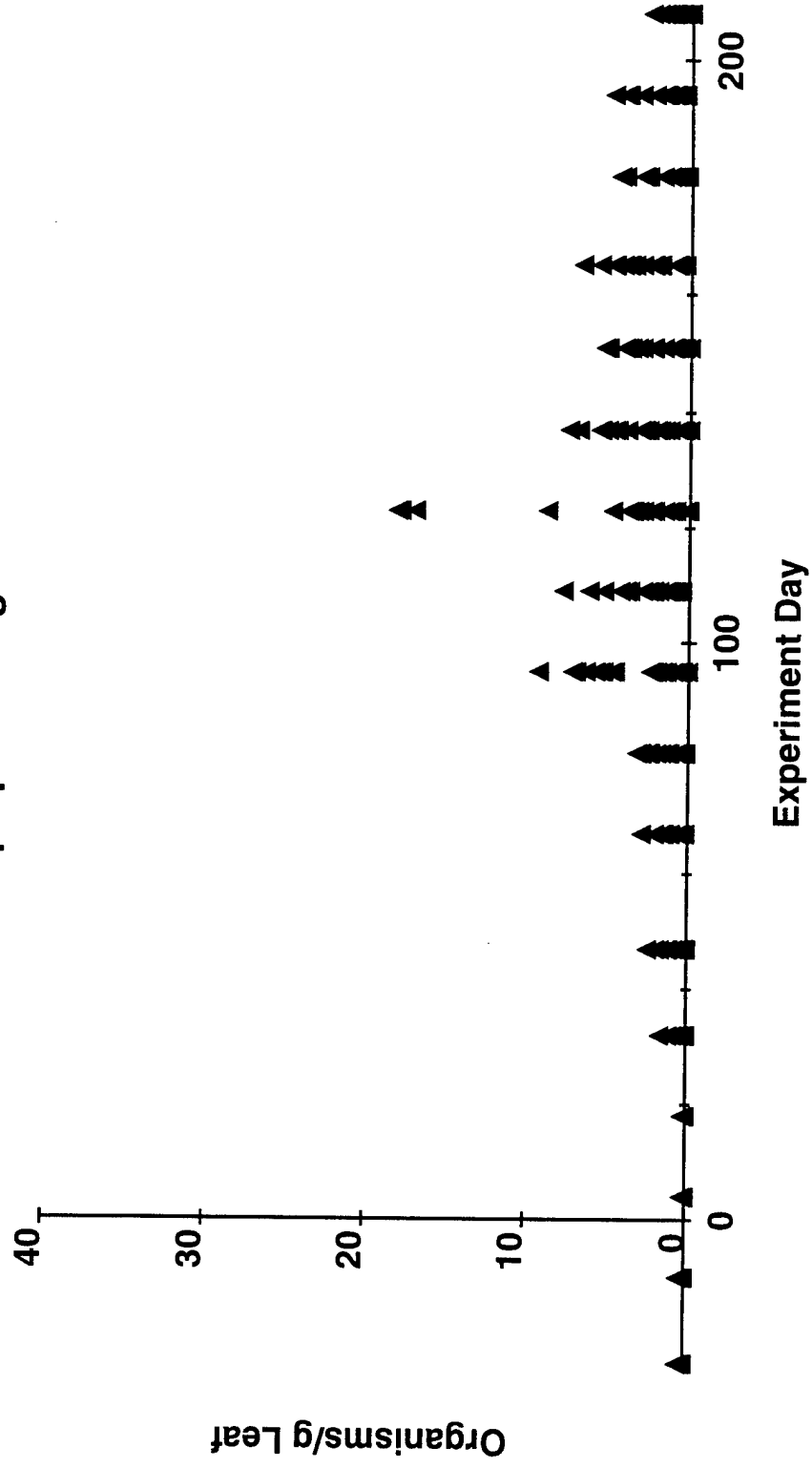
Figure 31b. Crustaceae
Amphipods - Mid-Dose
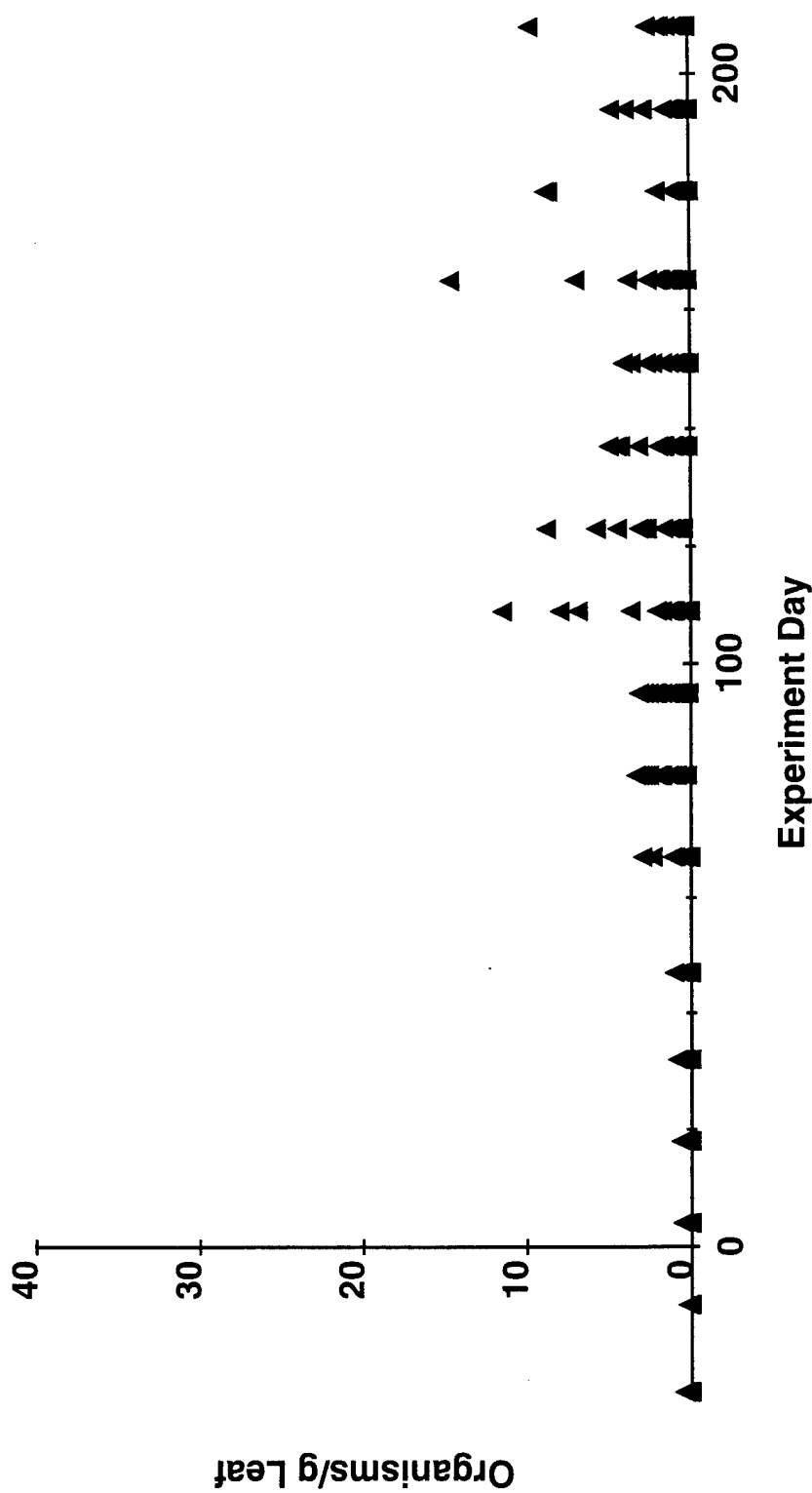
Figure 31c. Crustaceae
Amphipods - Low Dose
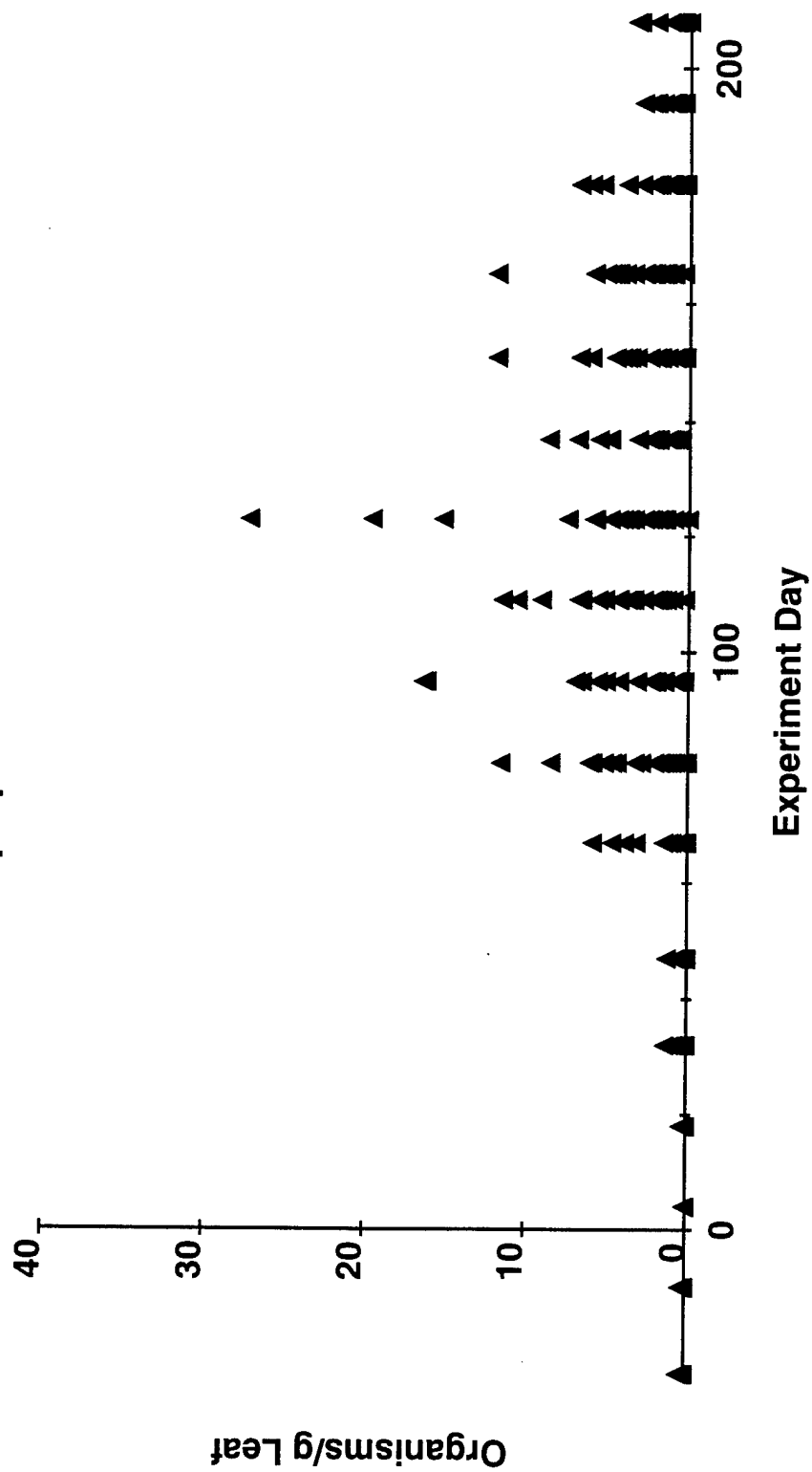
Figure 31d. Crustaceae
Amphipods - Undosed
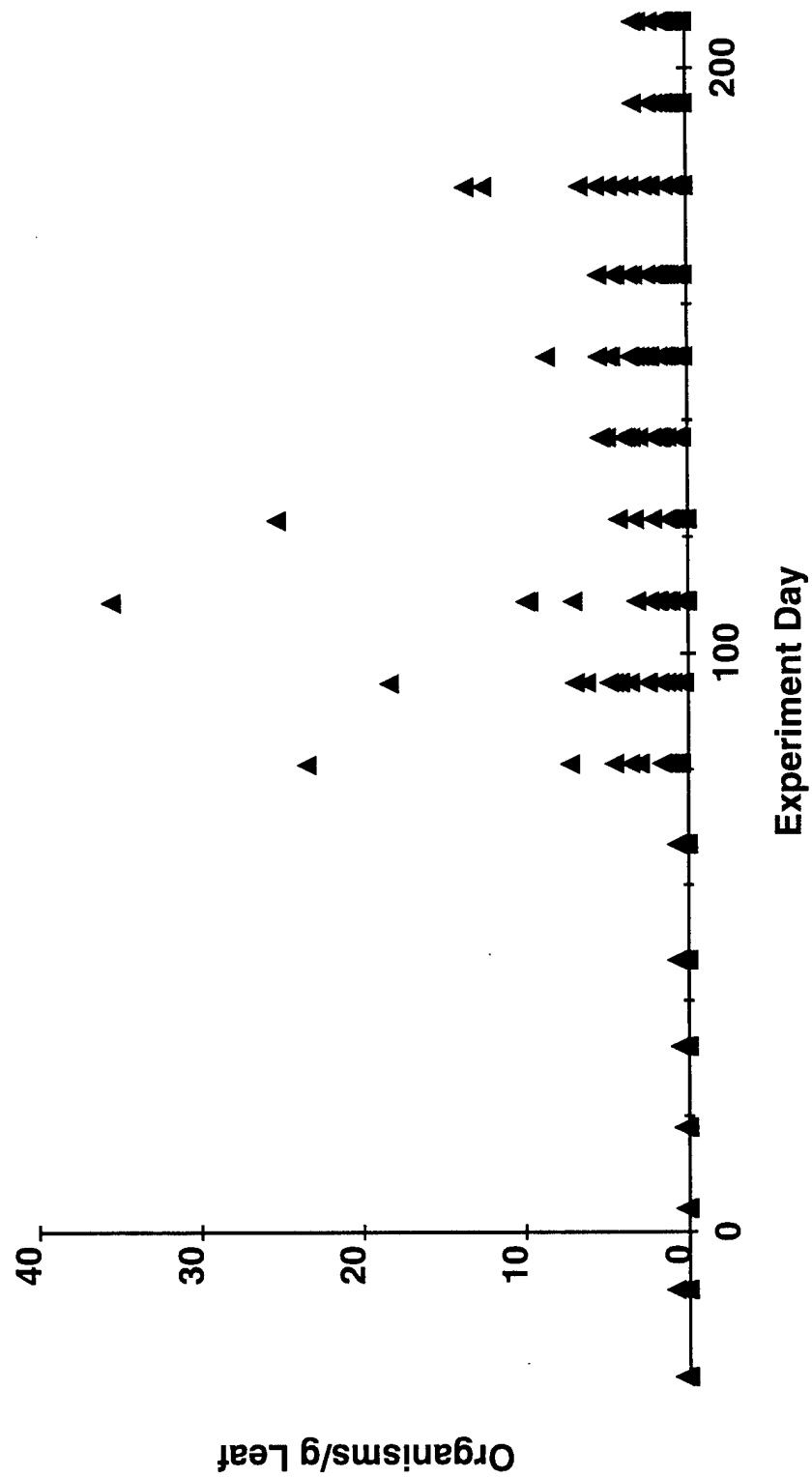
Figure 32a. Crustaceae
Copepods - High Dose
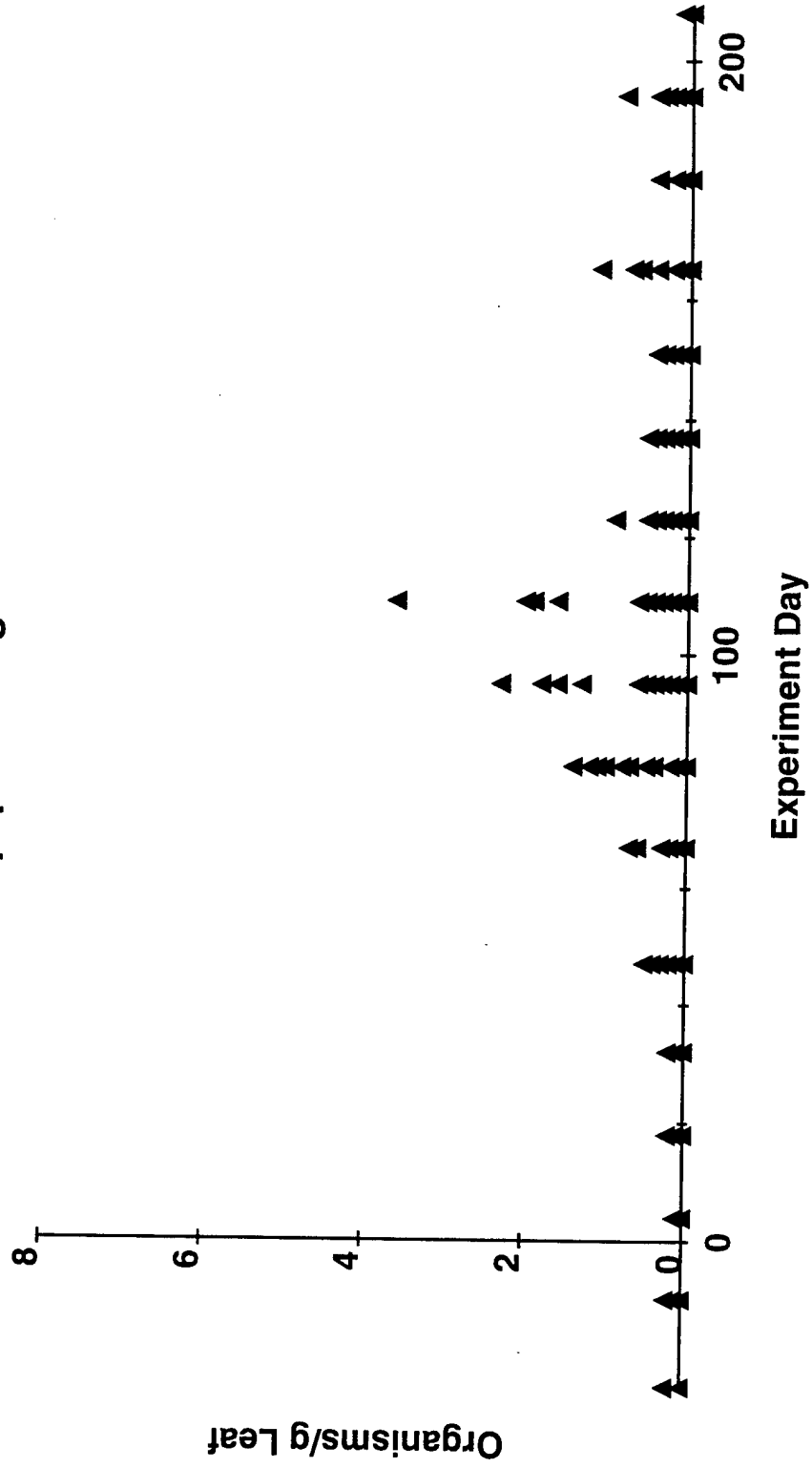
Figure 32b. Crustaceae
Copepods - Mid-Dose
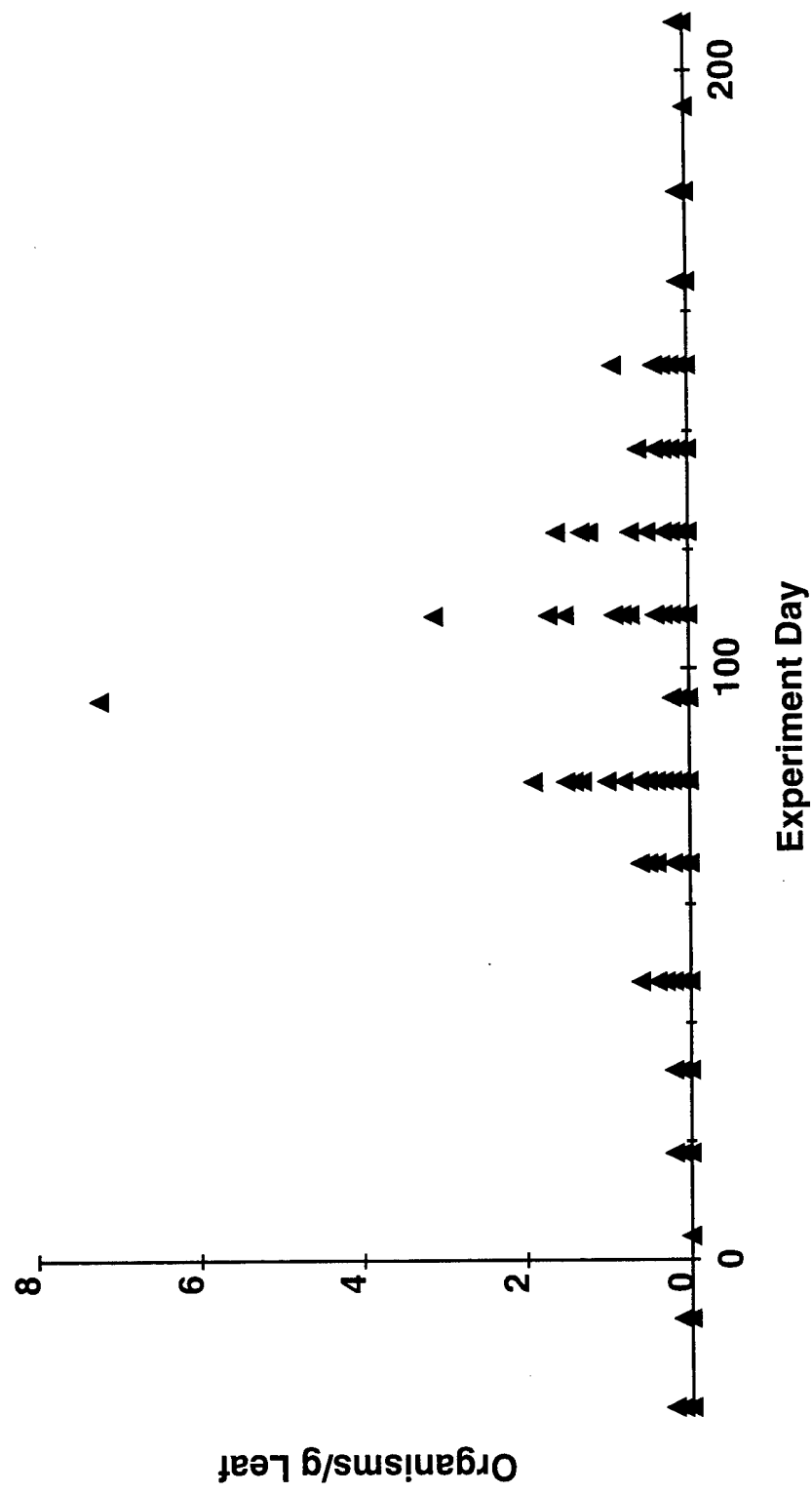
Figure 32c. Crustaceae
Copepods - Low Dose
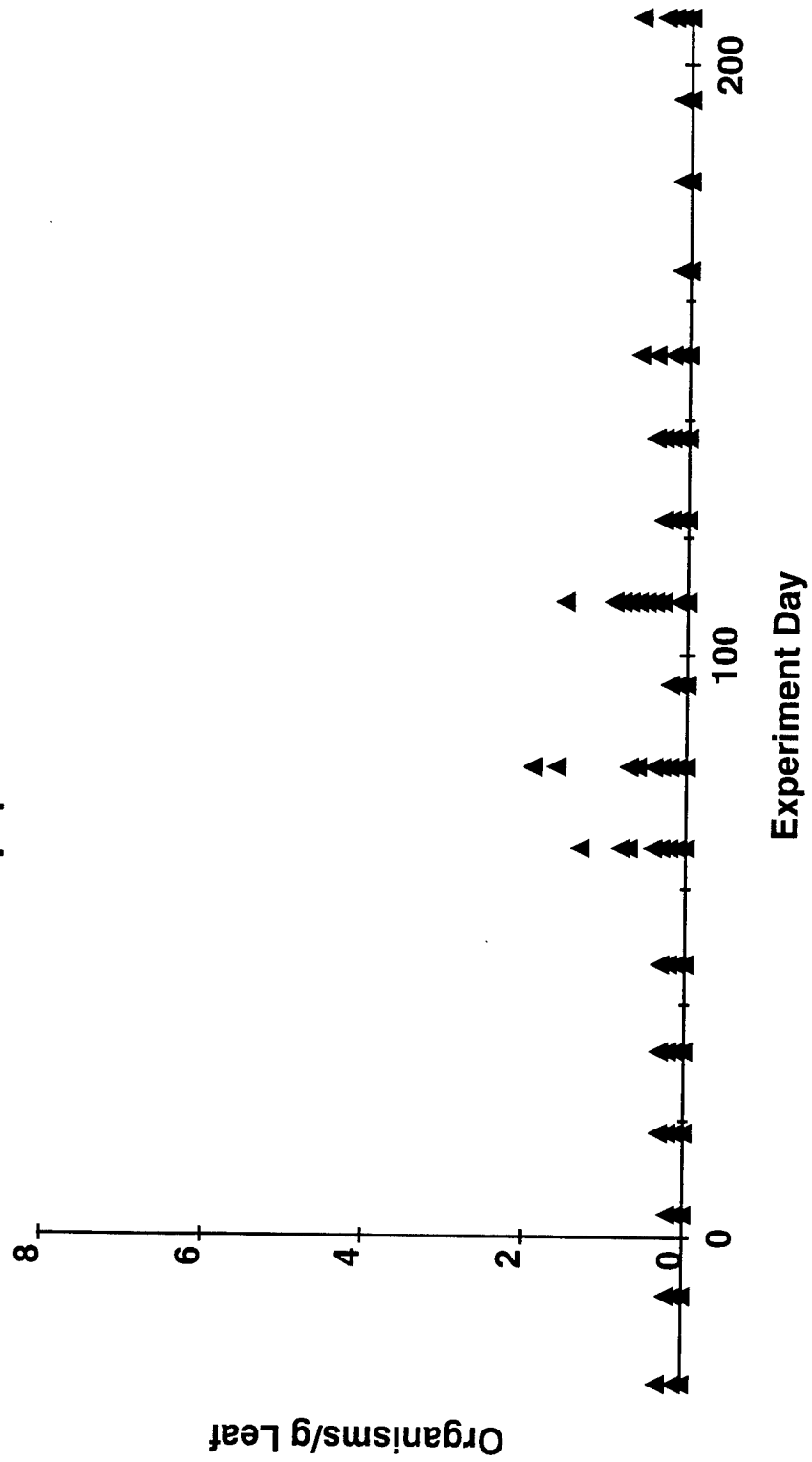
Figure 32d. Crustaceae
Copepods - Undosed
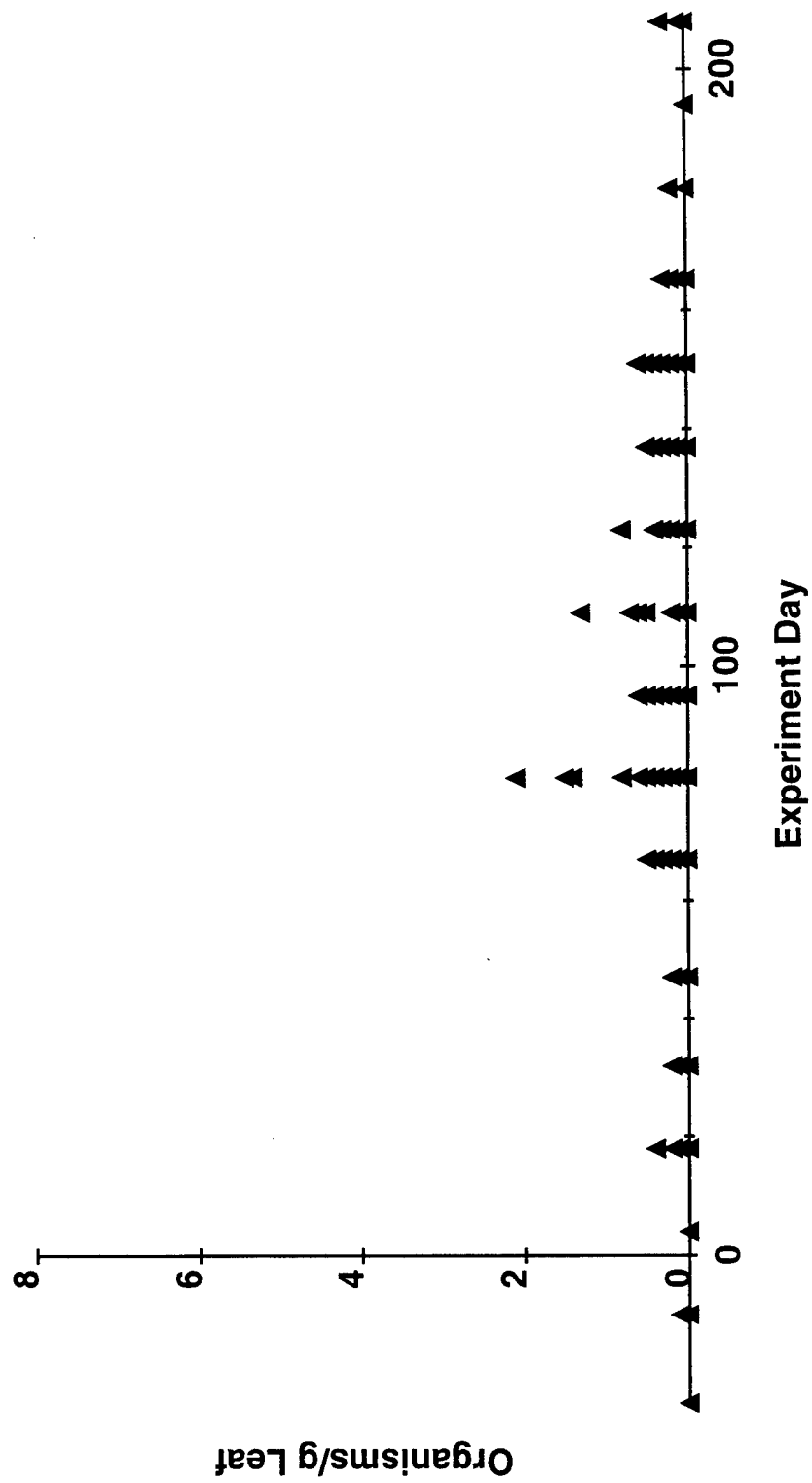
Figure 33a. Crustaceae
Daphnia - High Dose

Organisms/g Leaf

Experiment Day
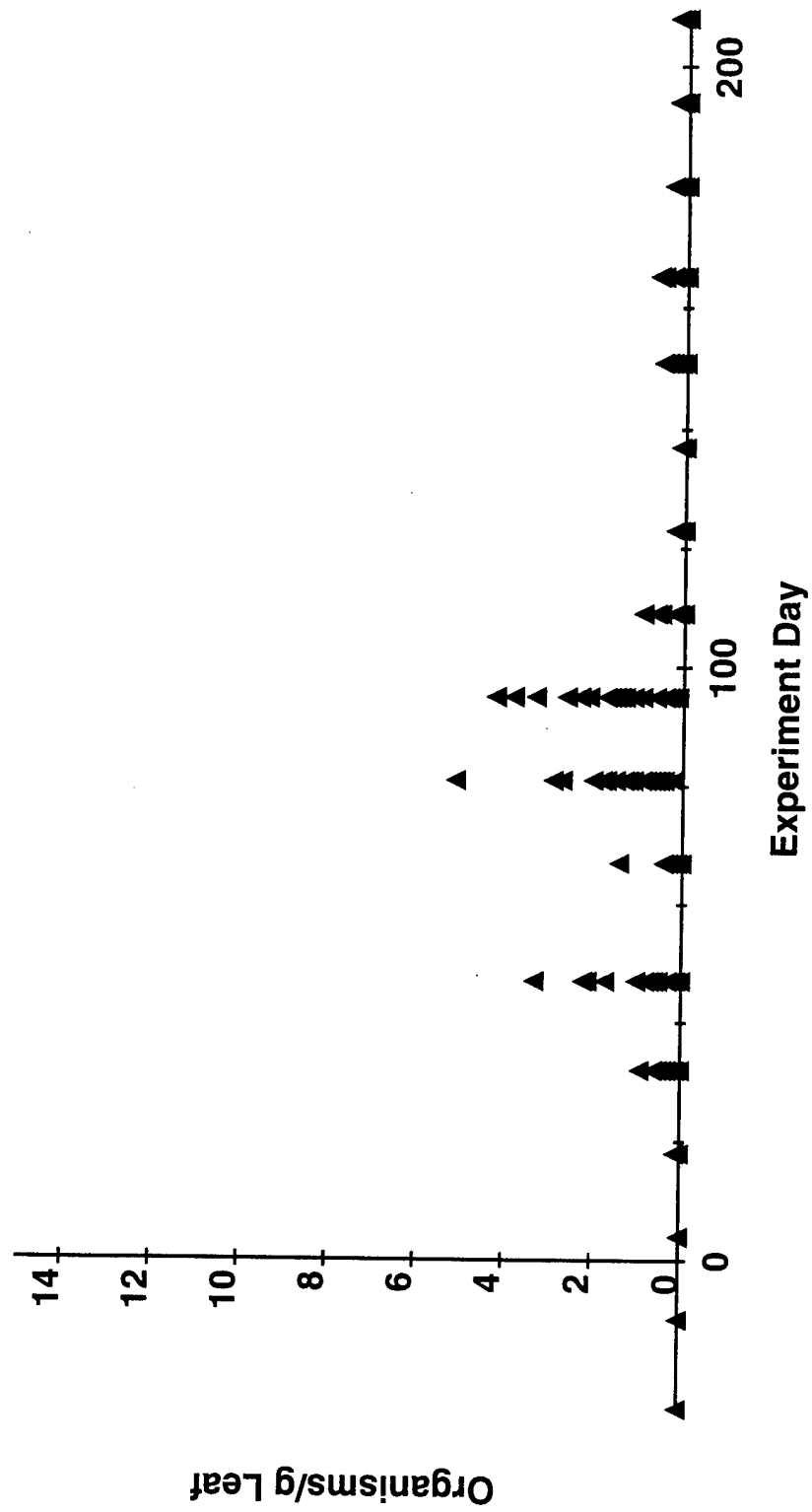
Figure 33b. Crustaceae
Daphnia - Mid-Dose
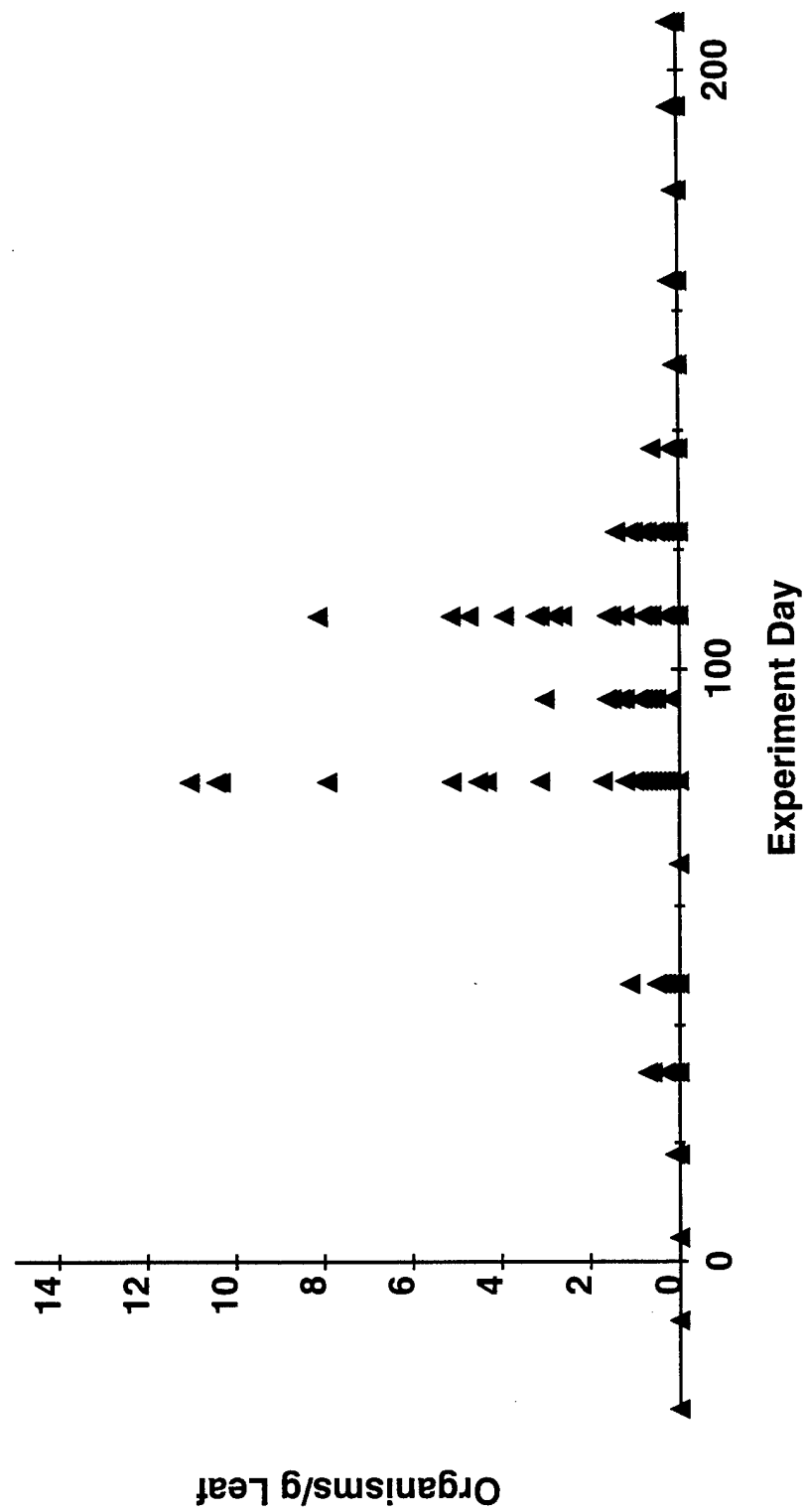
Figure 33c. Crustaceae
Daphnia - Low Dose

Organisms/g Leaf

Experiment Day
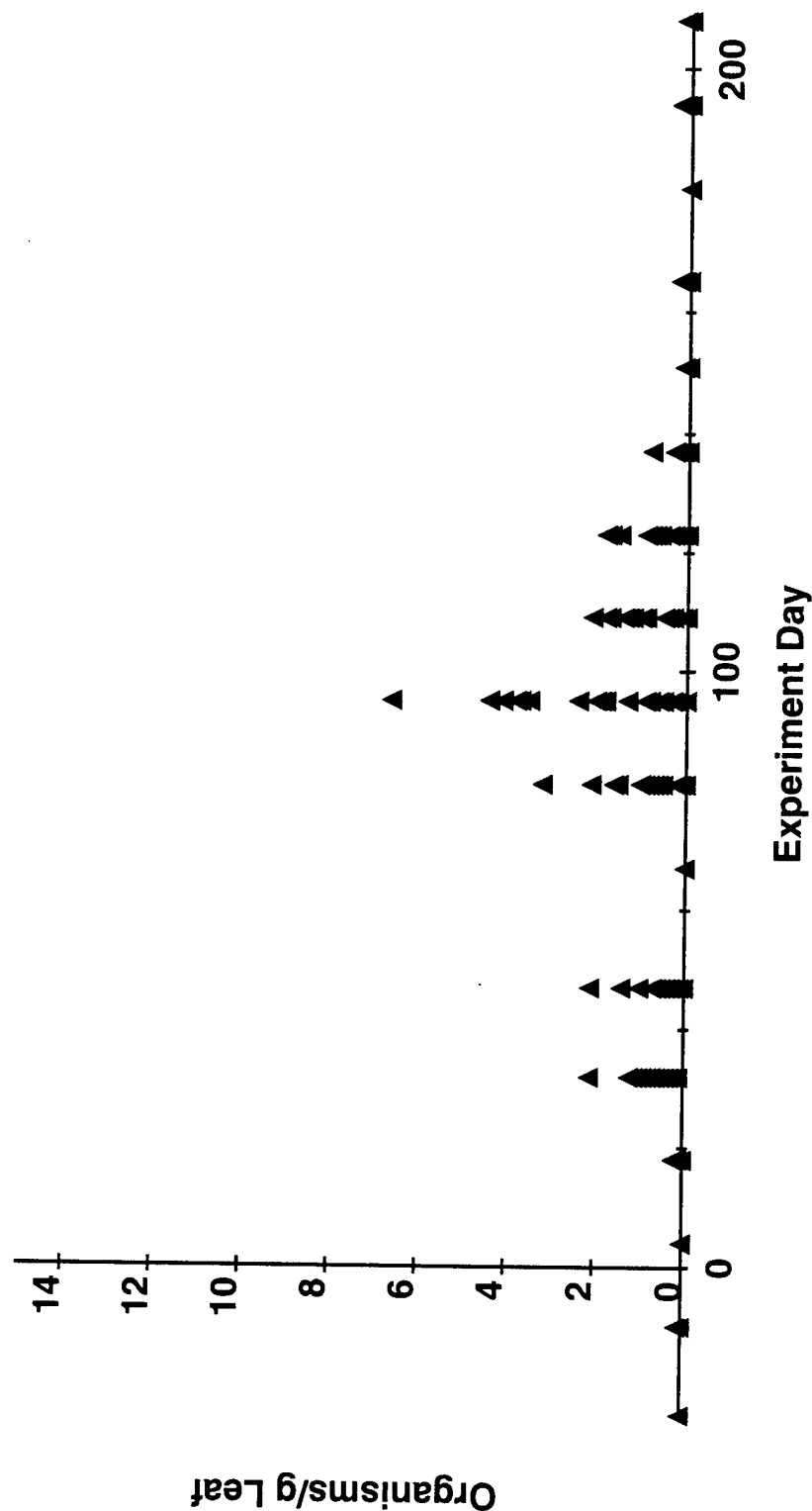
Figure 33d. Crustaceae
Daphnia - Undosed
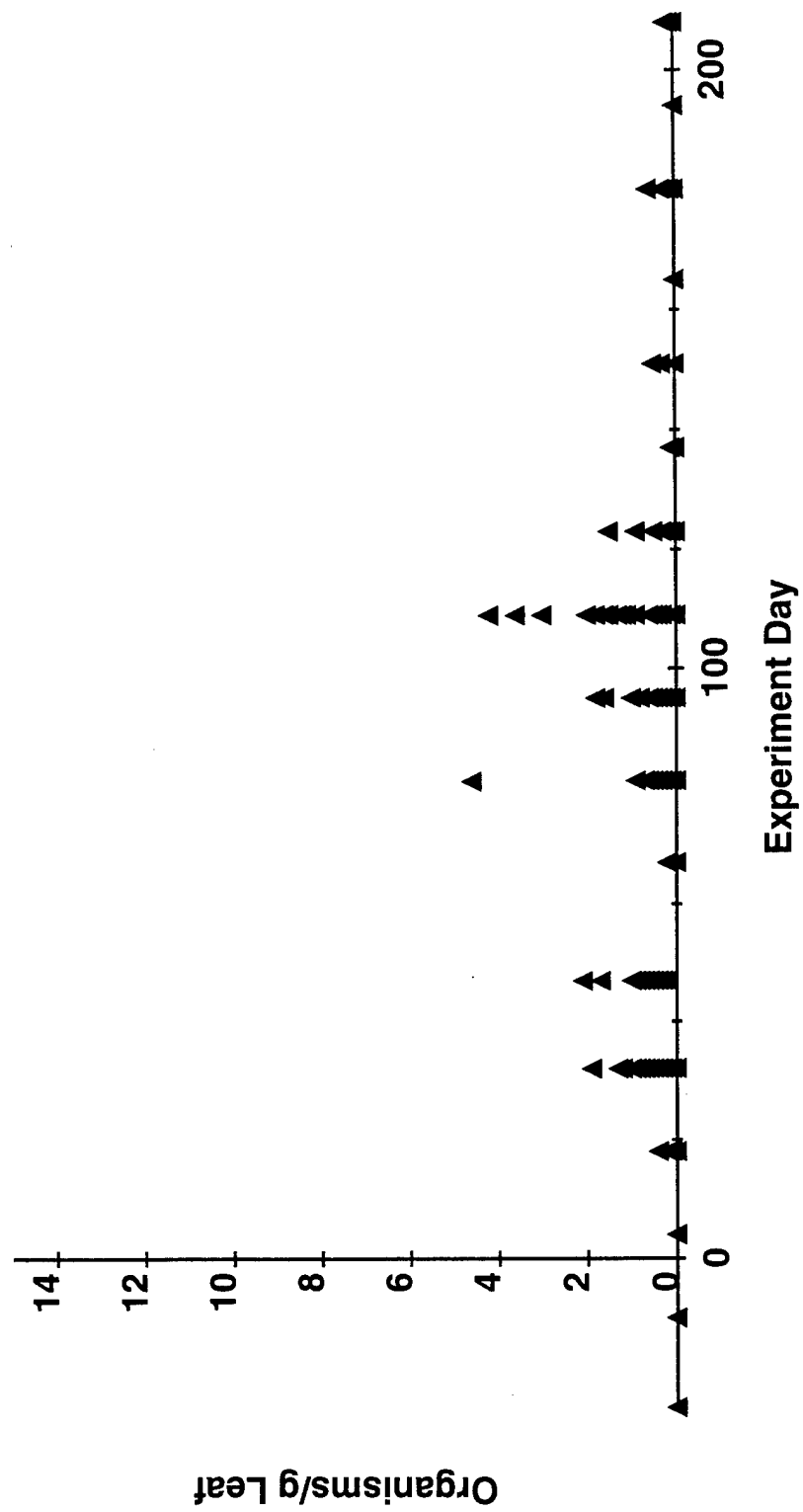
Figure 34a. Crustaceae
Ostracods - High Dose
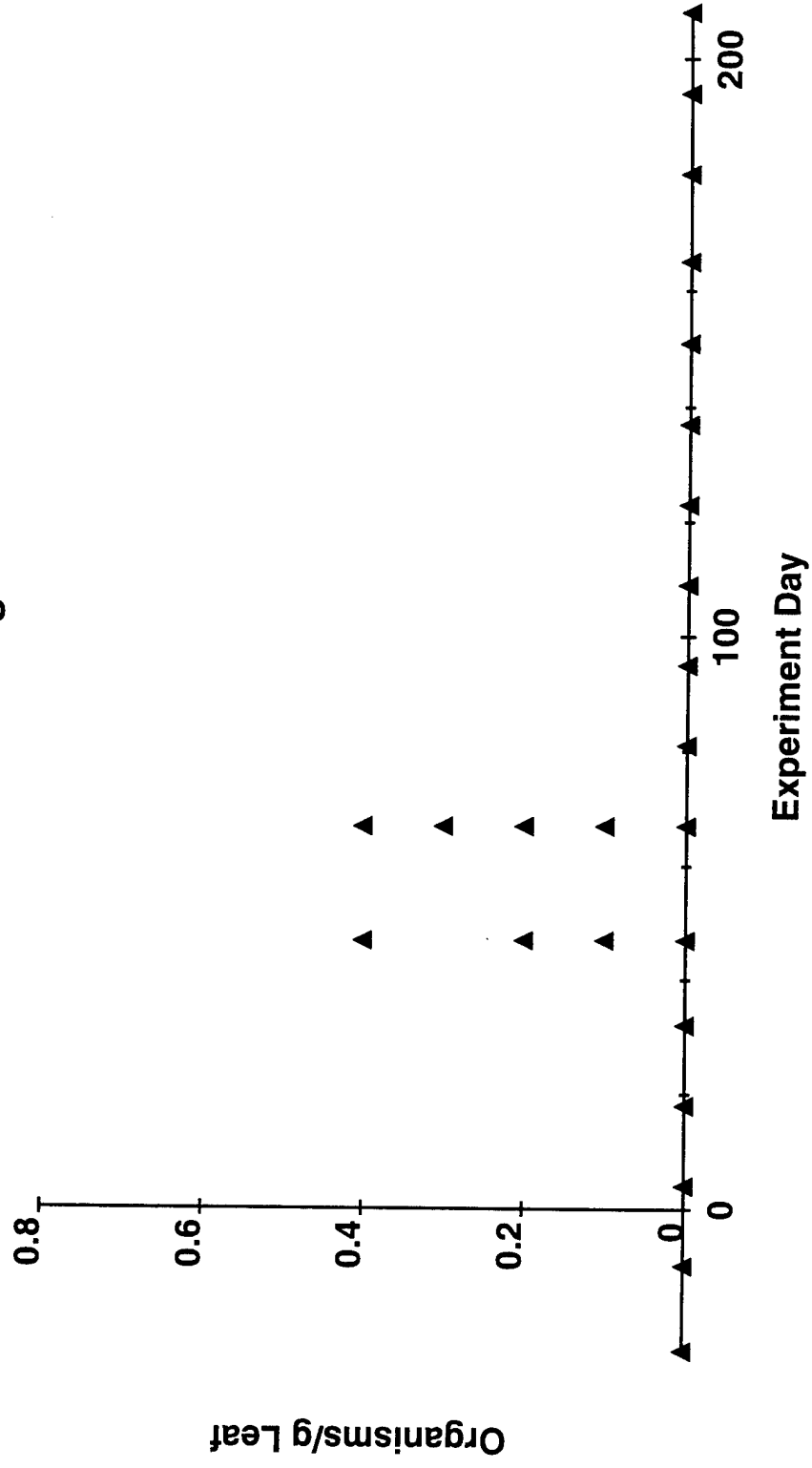
Figure 34b. Crustaceae
Ostracods - Mid-Dose
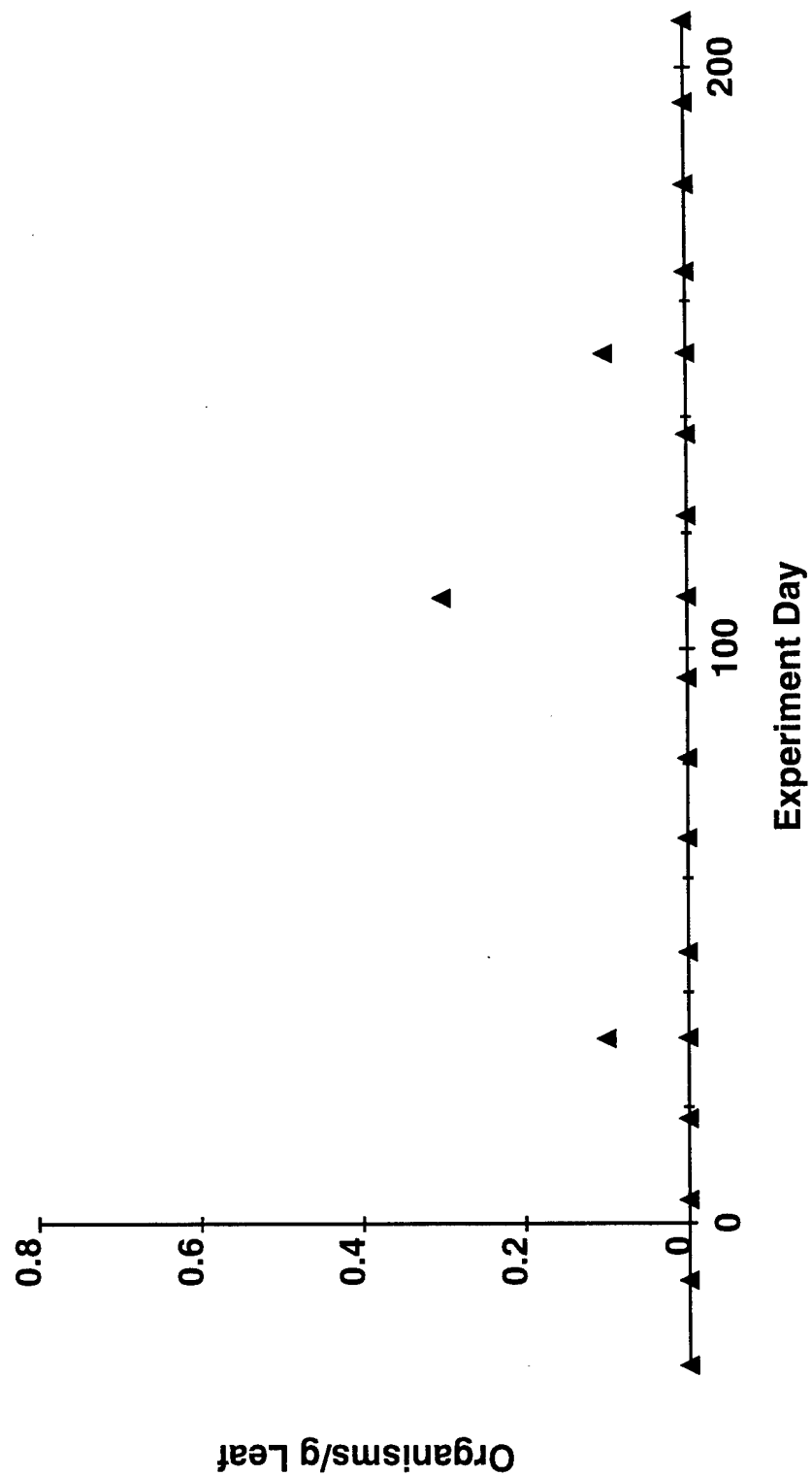
Figure 34c. Crustaceae
Ostracods - Low Dose
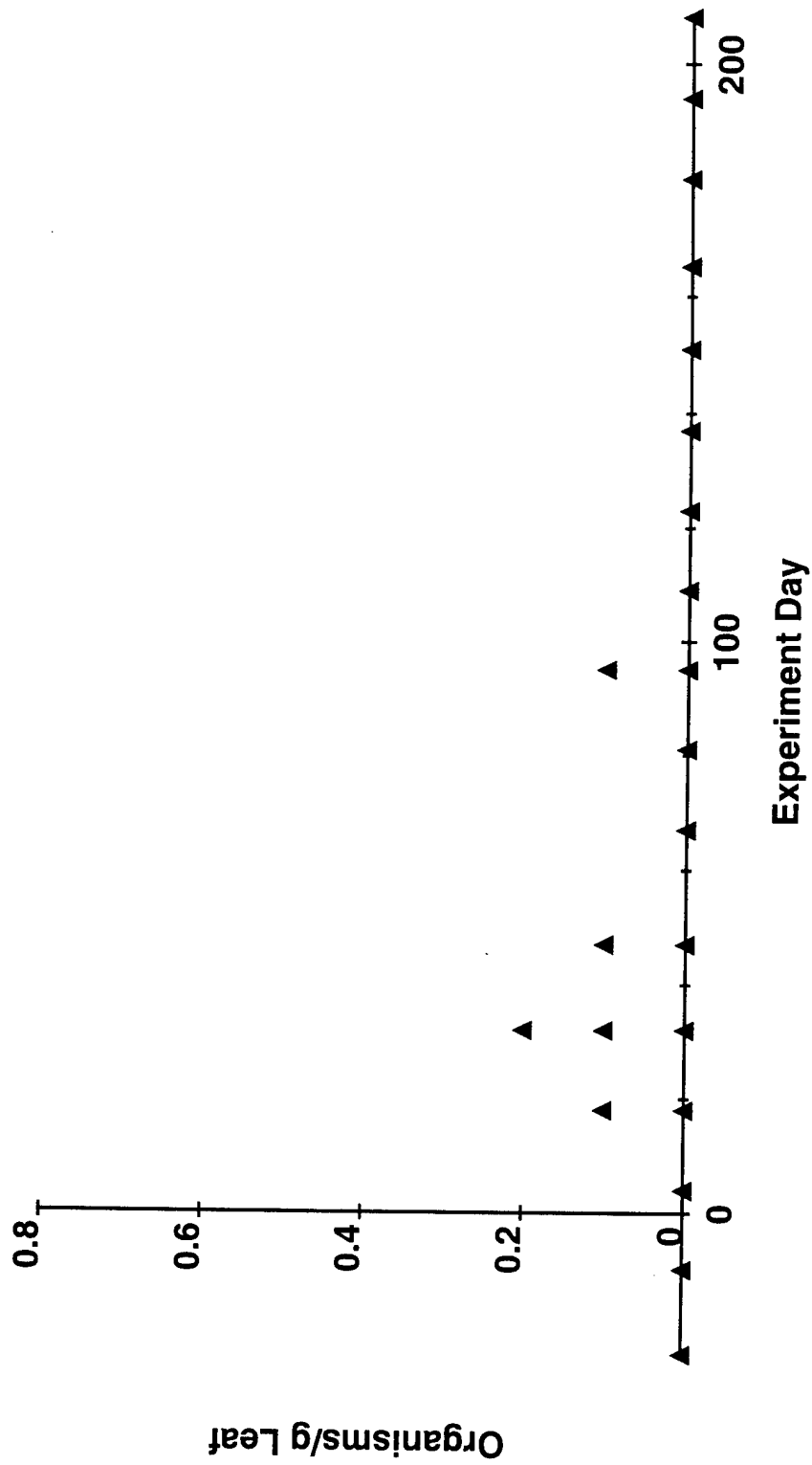
Figure 34c. Crustaceae
Ostracods - Low Dose
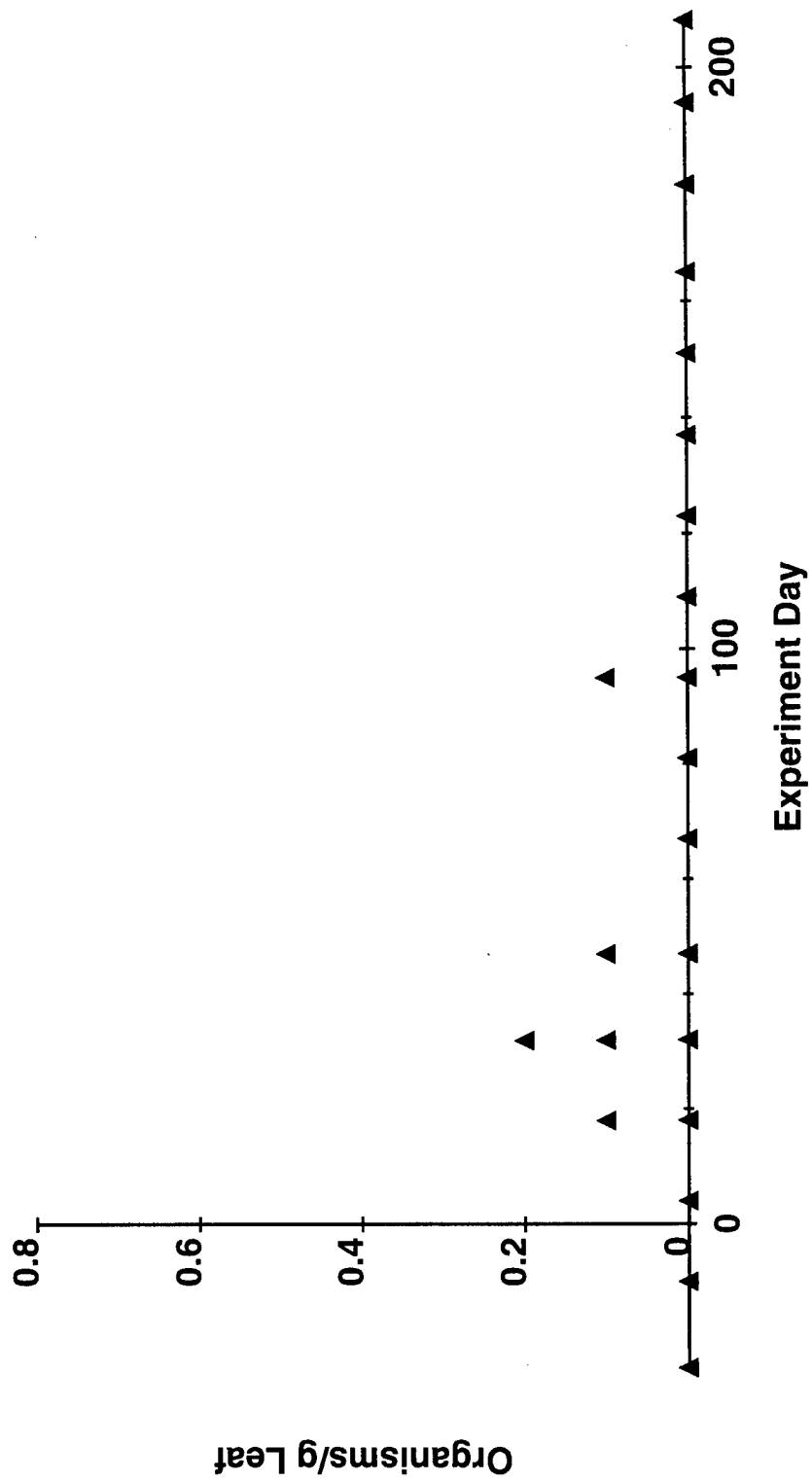
Figure 34d. Crustaceae
Ostracods - Undosed

Organisms/g Leaf

Experiment Day
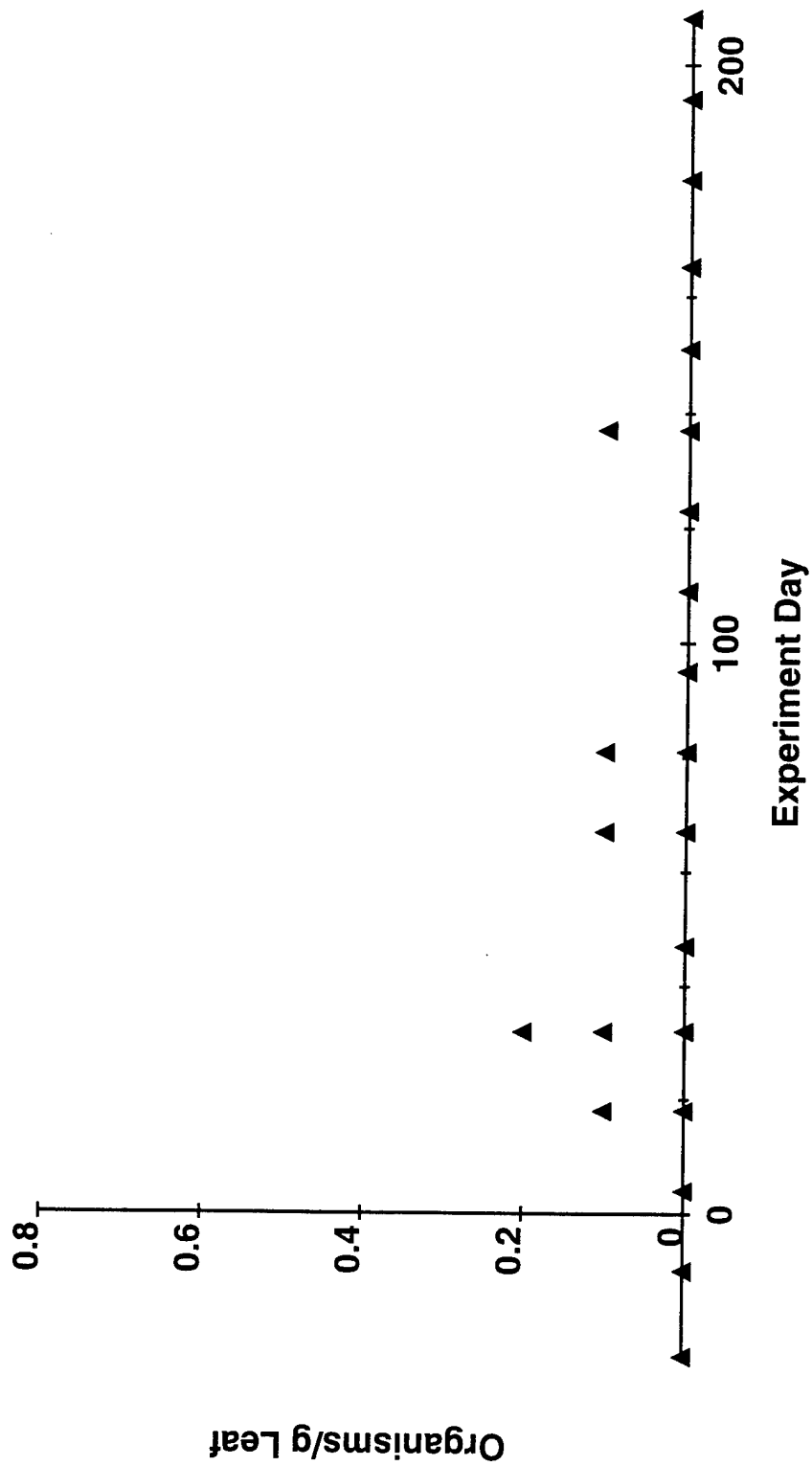
Figure 35a. Insects
Diptera, Chironomids - High Dose
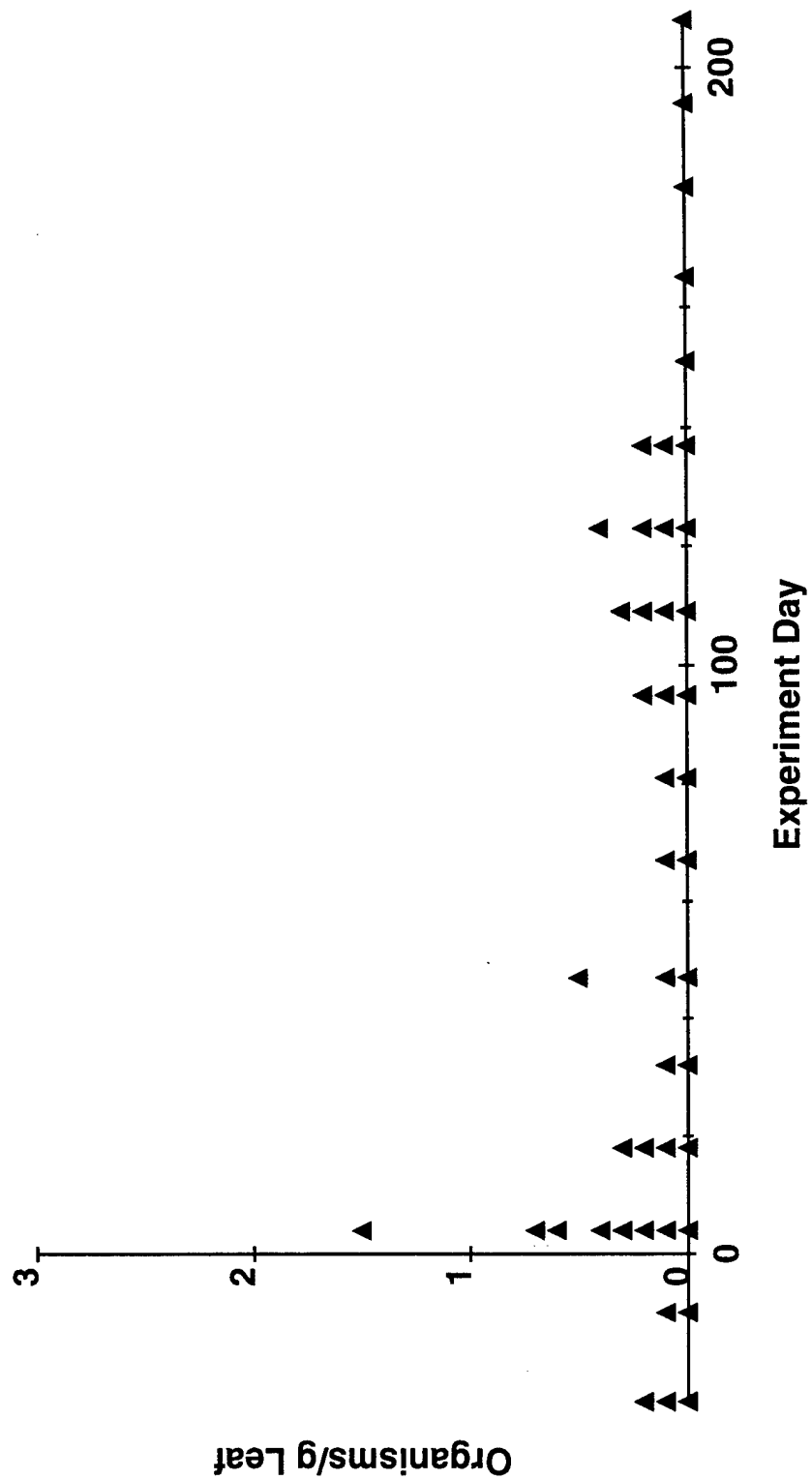
Figure 35b. Insects
Diptera, Chironomids - Mid-Dose
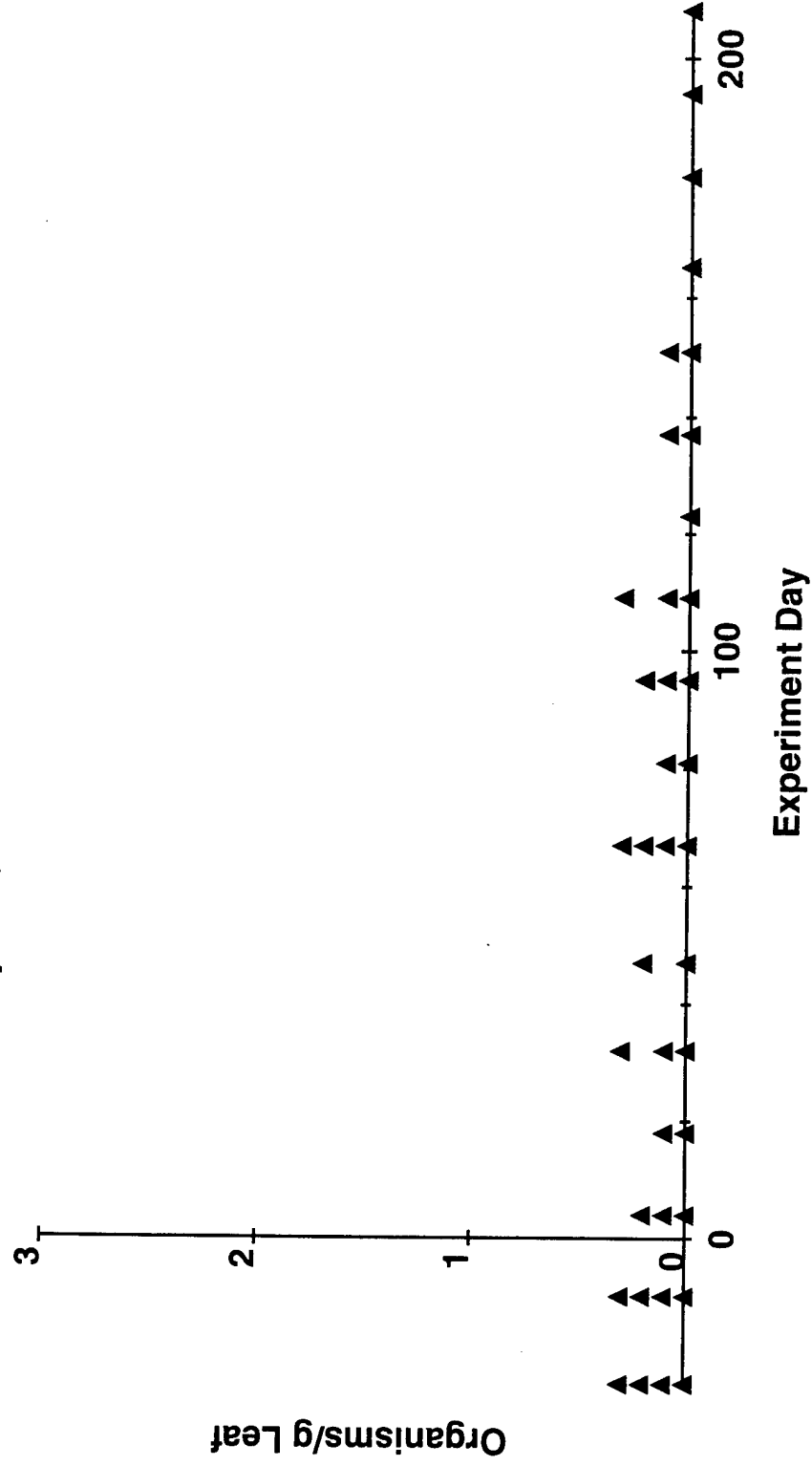
Figure 35c. Insects
Diptera, Chironomids - Low Dose
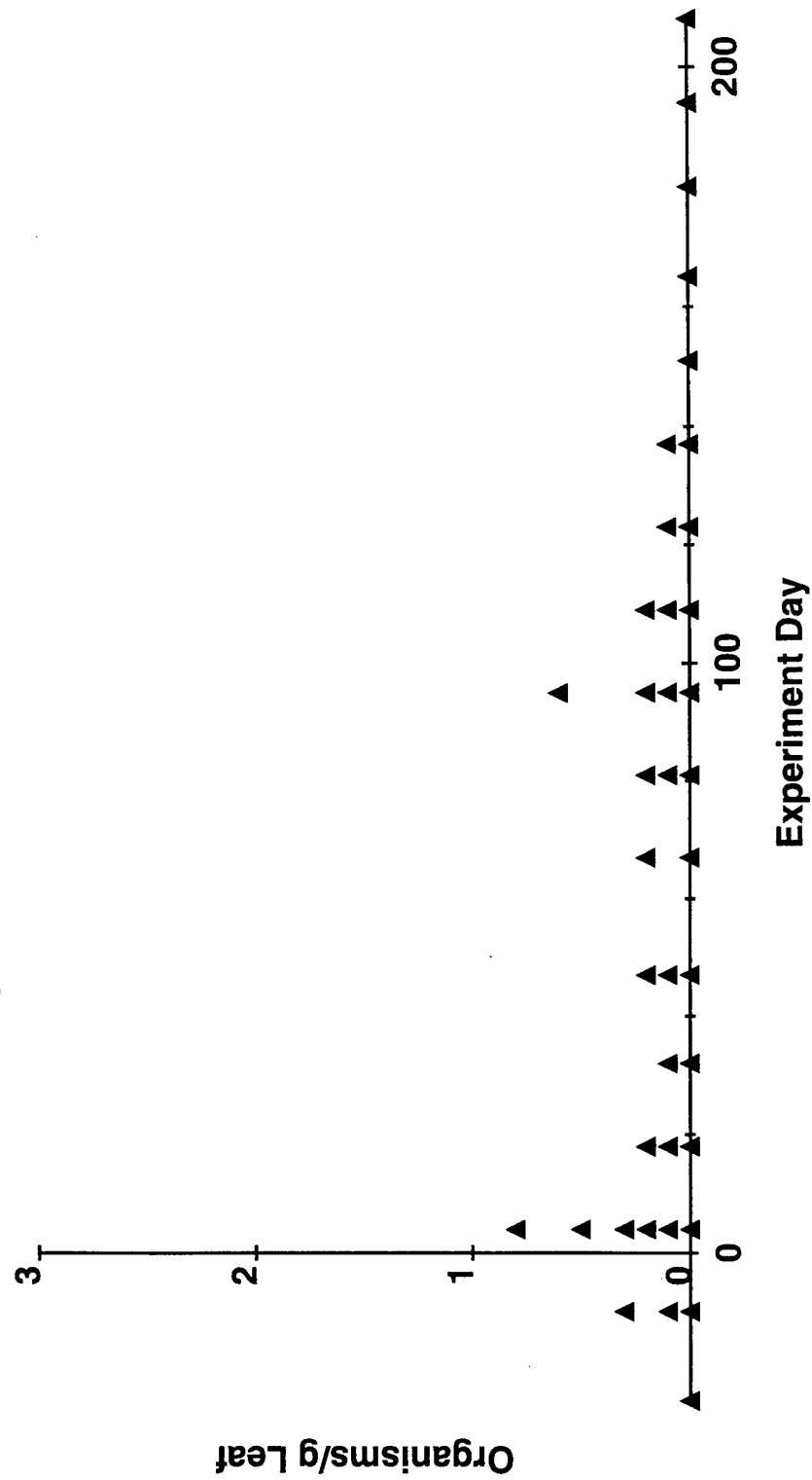
Figure 35d. Insects
Diptera, Chironomids - Undosed
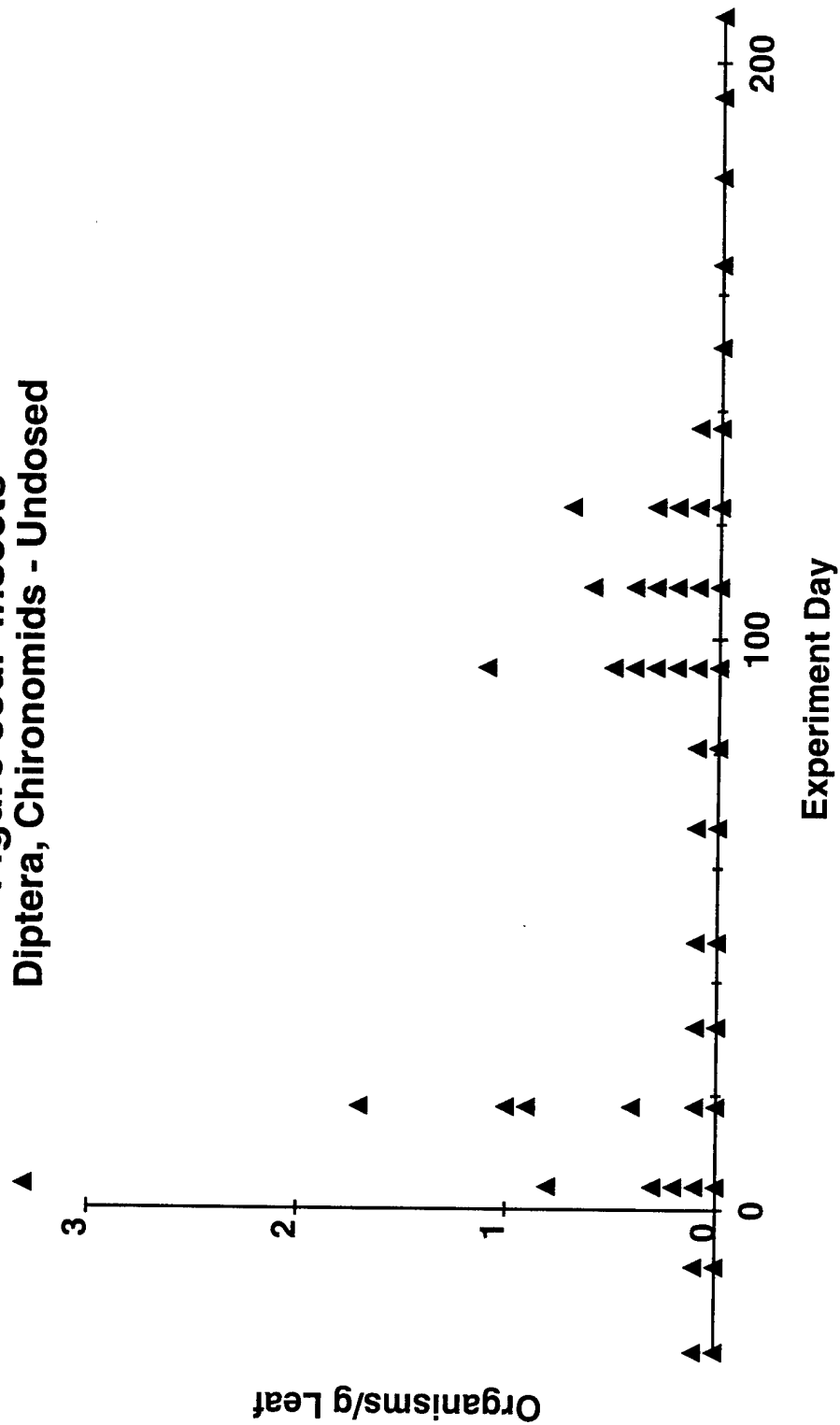
Figure 36a. Insects
Diptera, Mosquitoes - High Dose
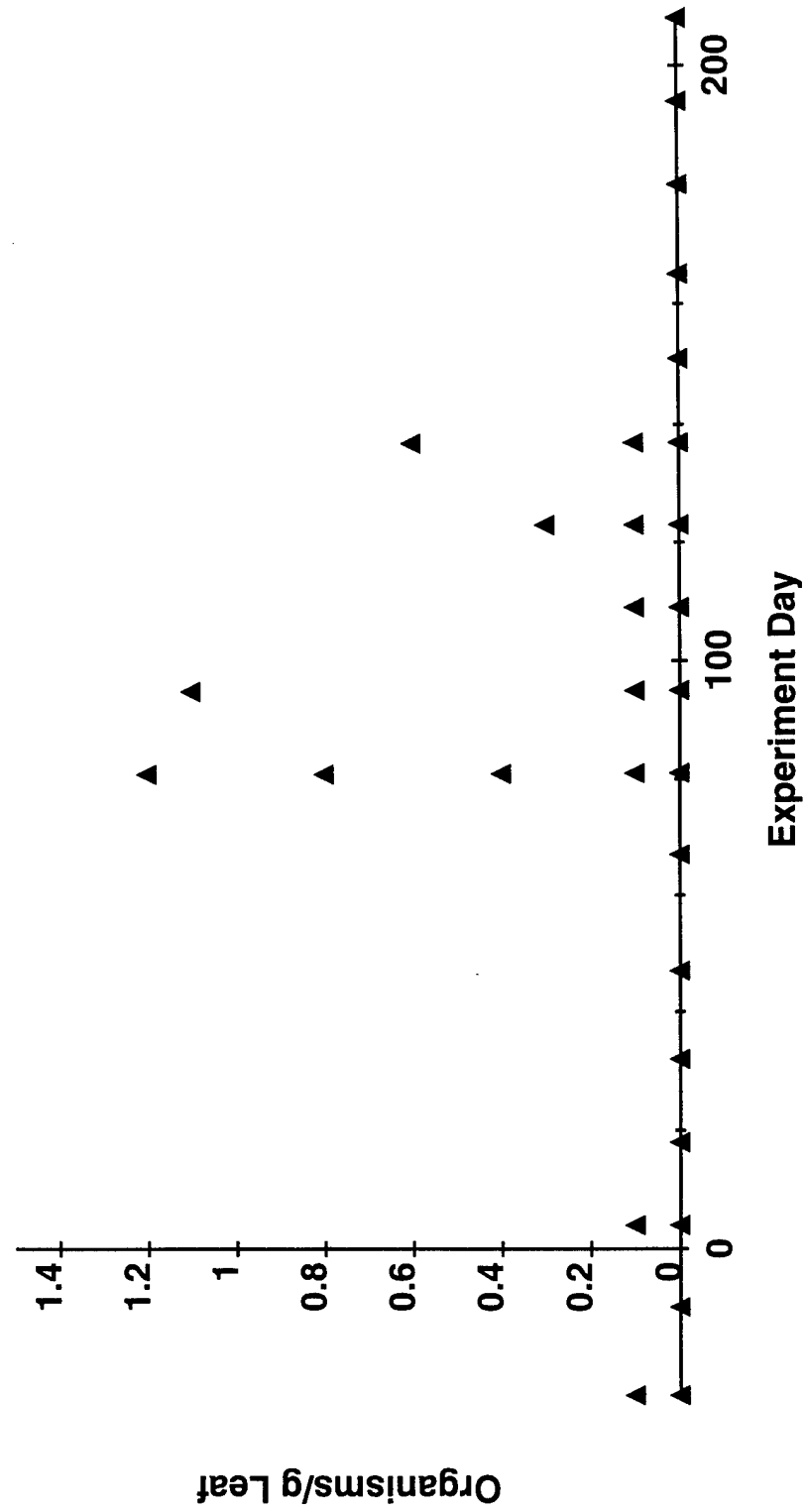
Figure 36b. Insects
Diptera, Mosquitoes - Mid-Dose
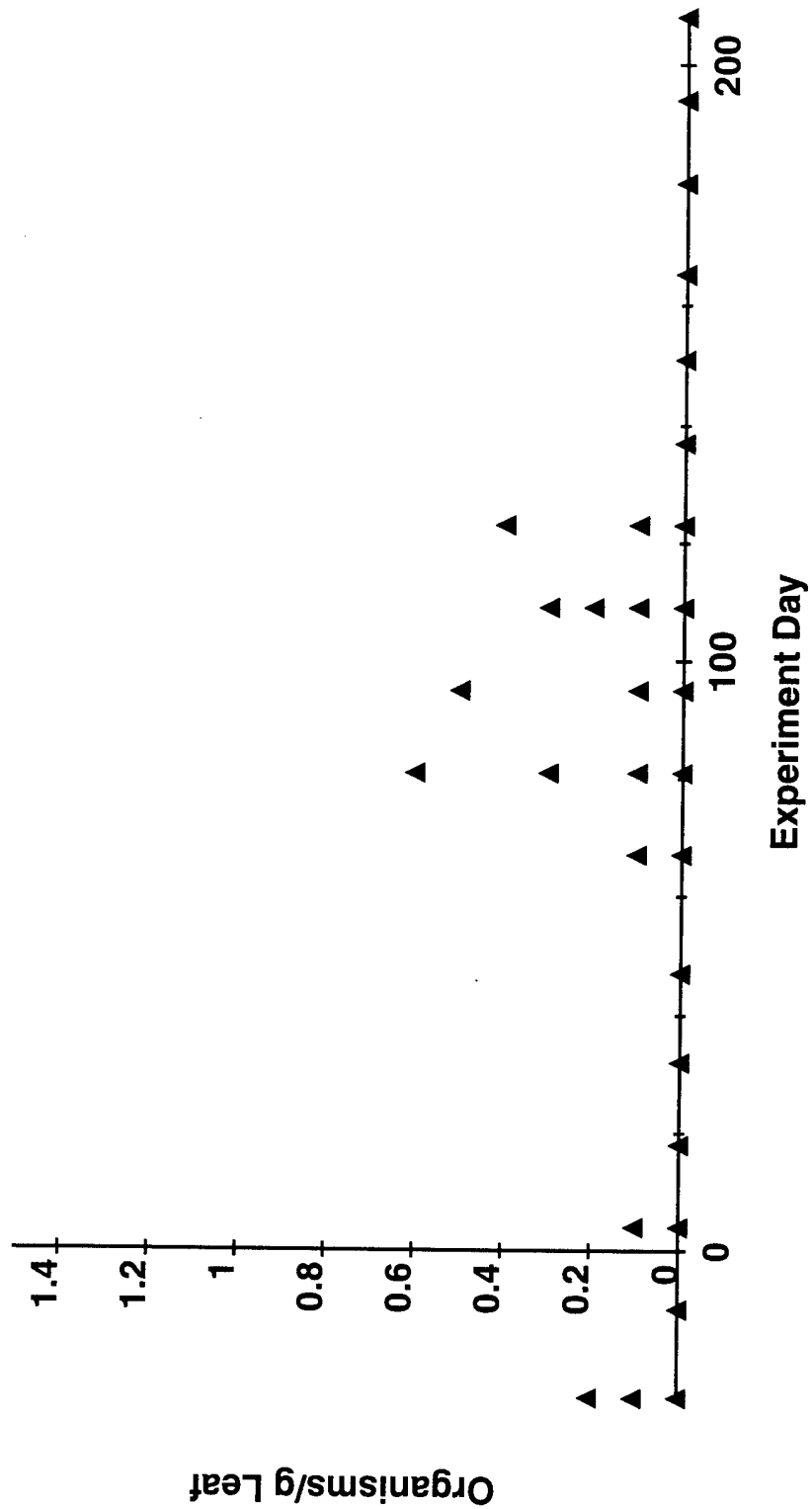
Figure 36c. Insects
Diptera, Mosquitoes - Low Dose
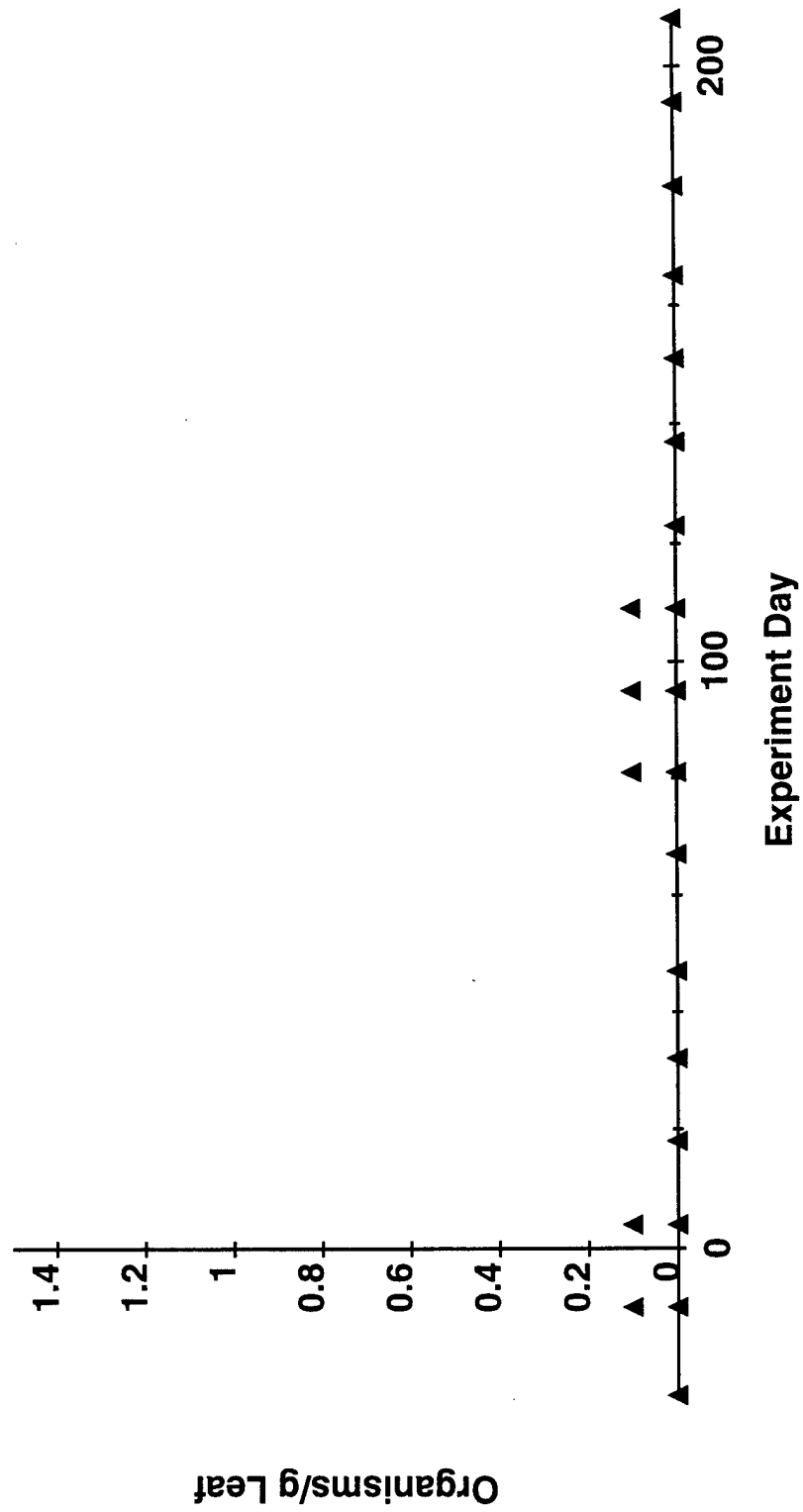
Figure 36d. Insects
Diptera, Mosquitoes - Undosed
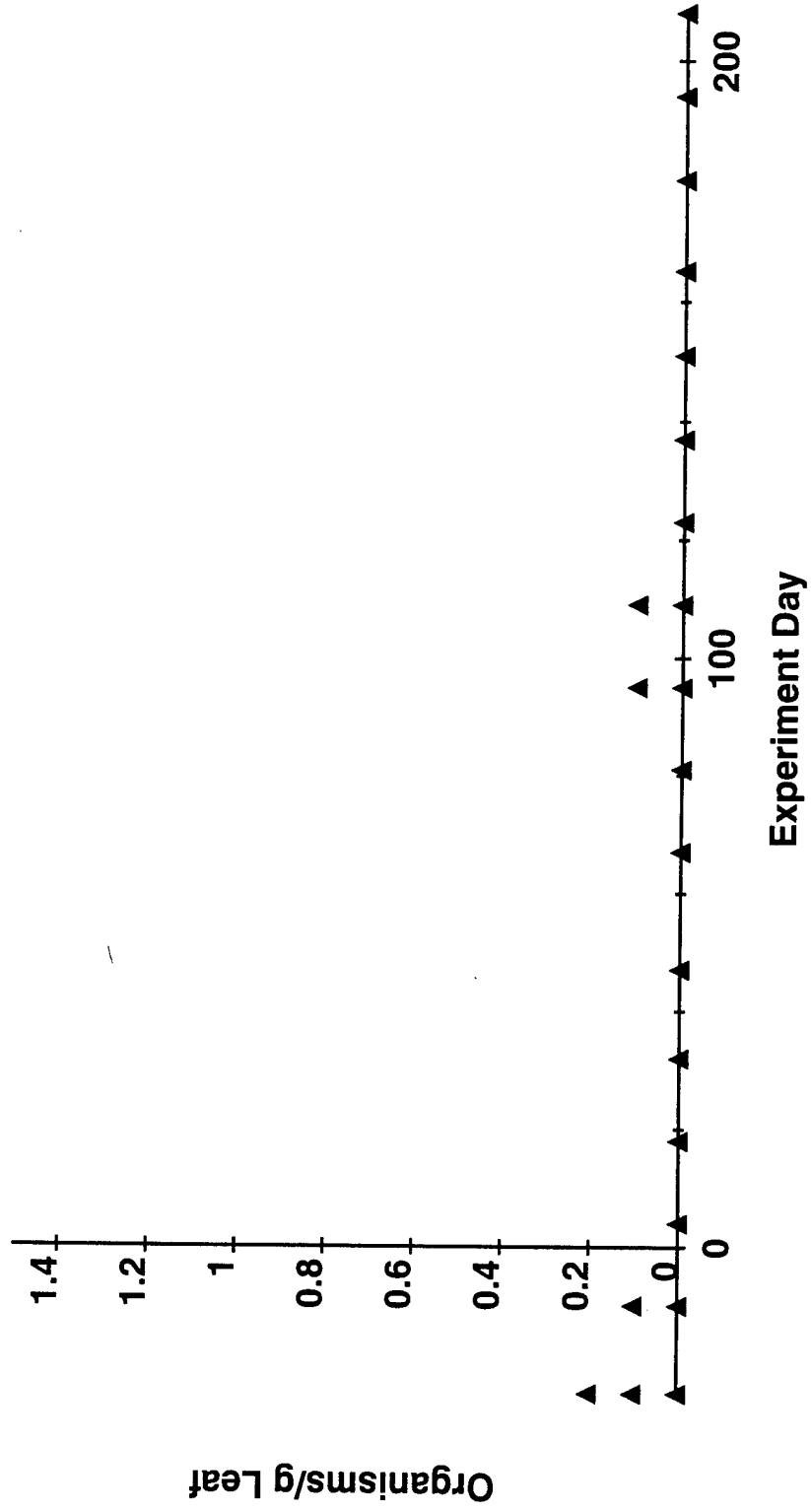
Figure 37a. Insects
Ephemeroptera - High Dose

Organisms/g Leaf

Experiment Day
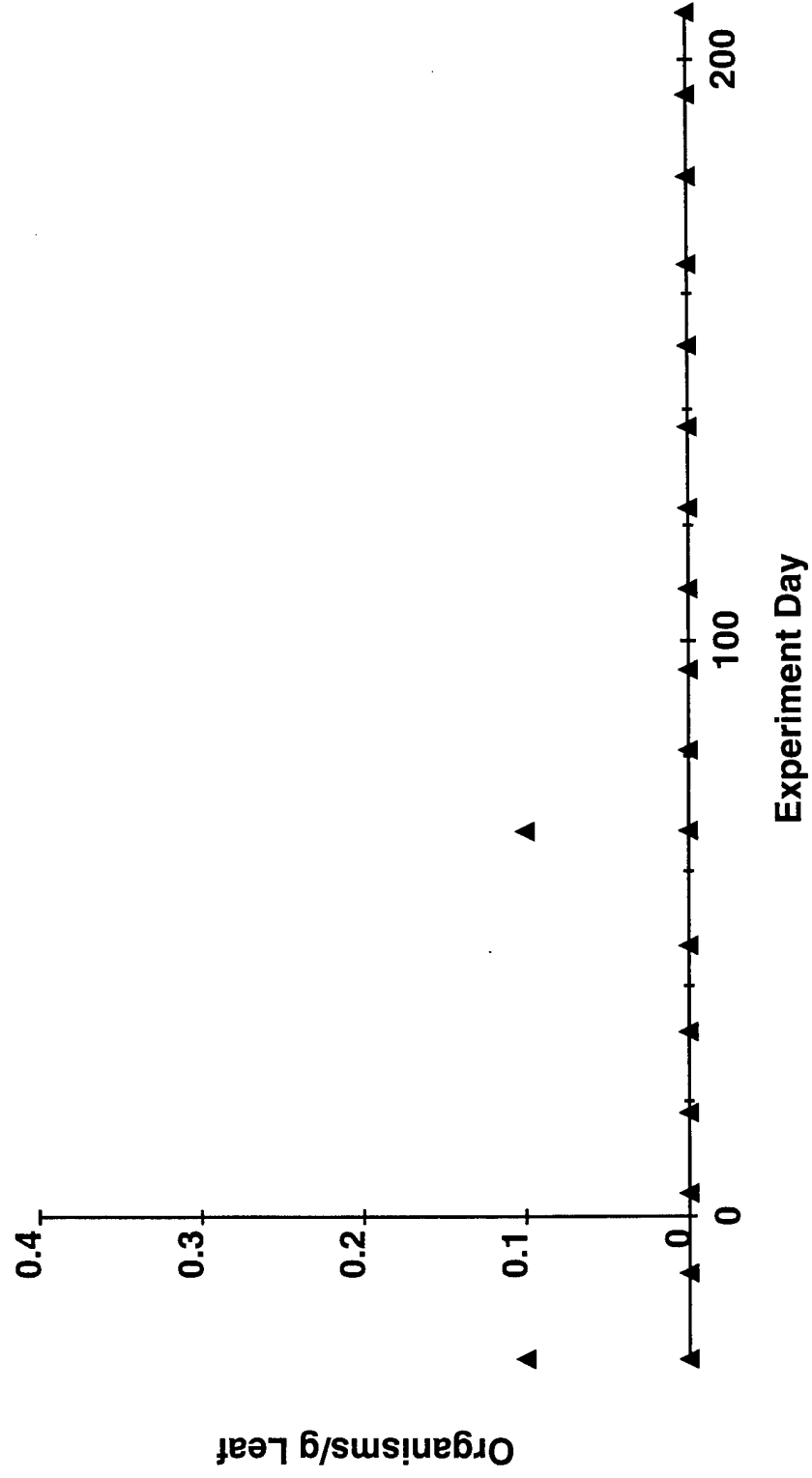
Figure 37b. Insects
Ephemeroptera - Mid-Dose

Organisms/g Leaf

Experiment Day
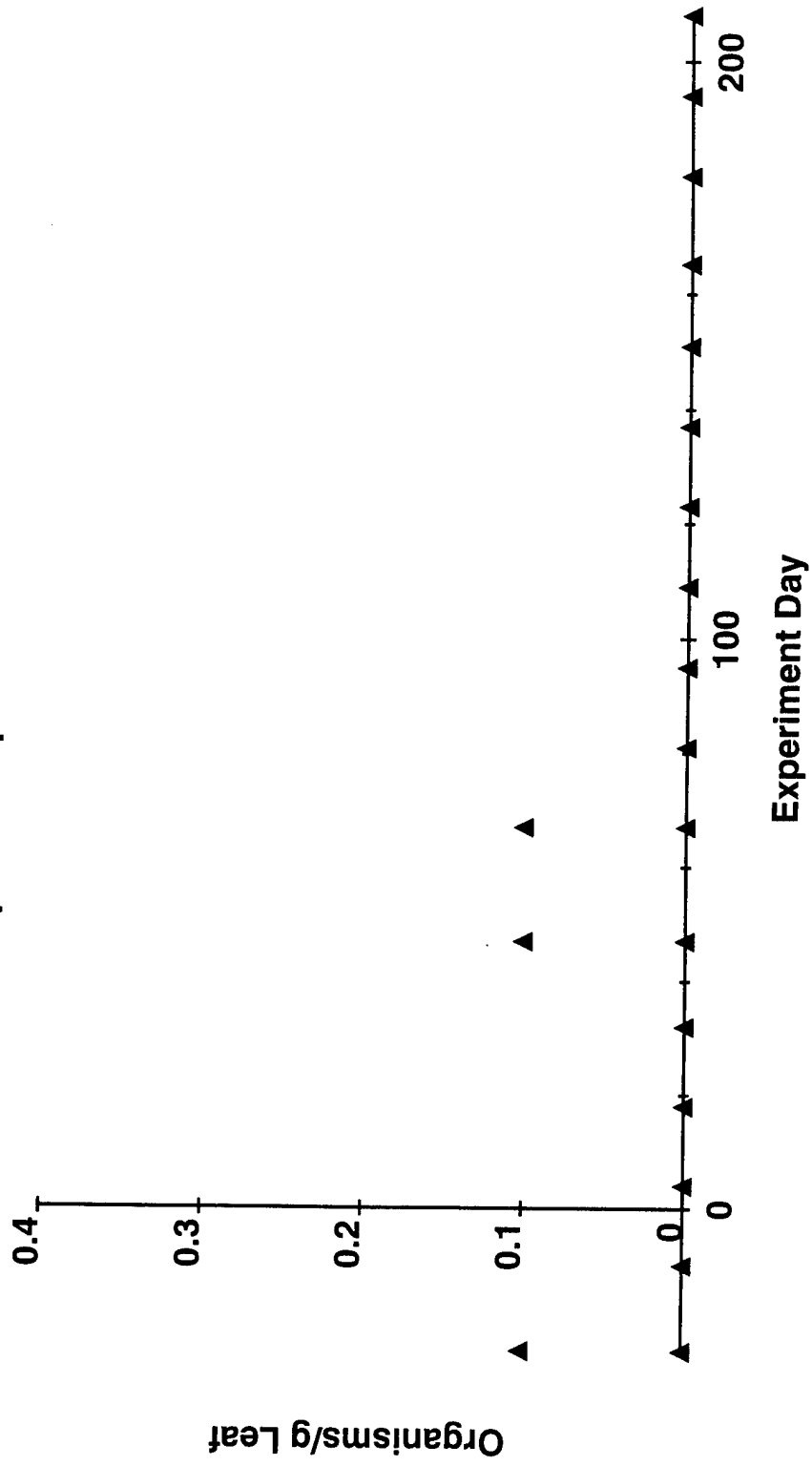
Figure 37c. Insects
Ephemeroptera - Low Dose
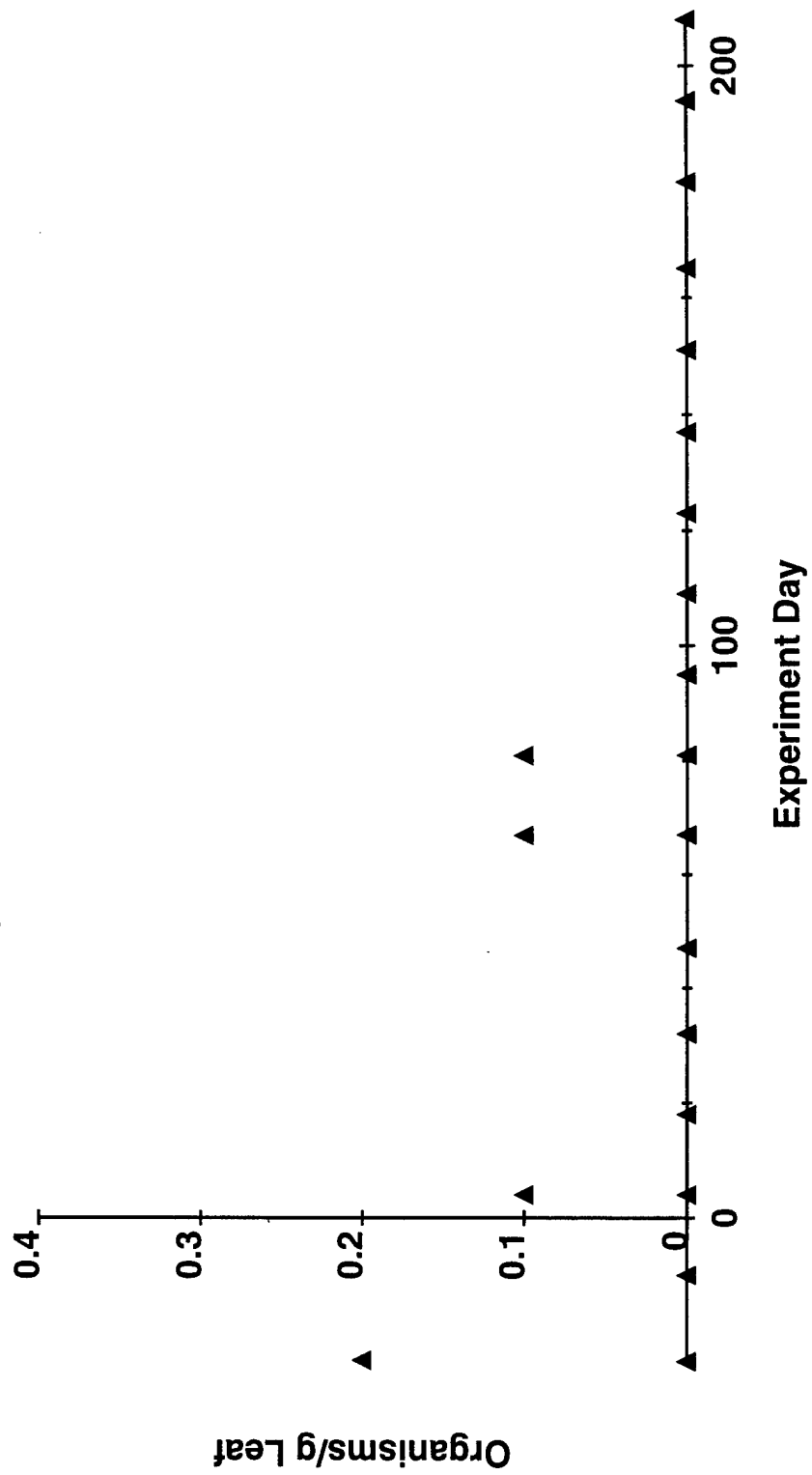
Figure 37d. Insects
Ephemeroptera - Undosed
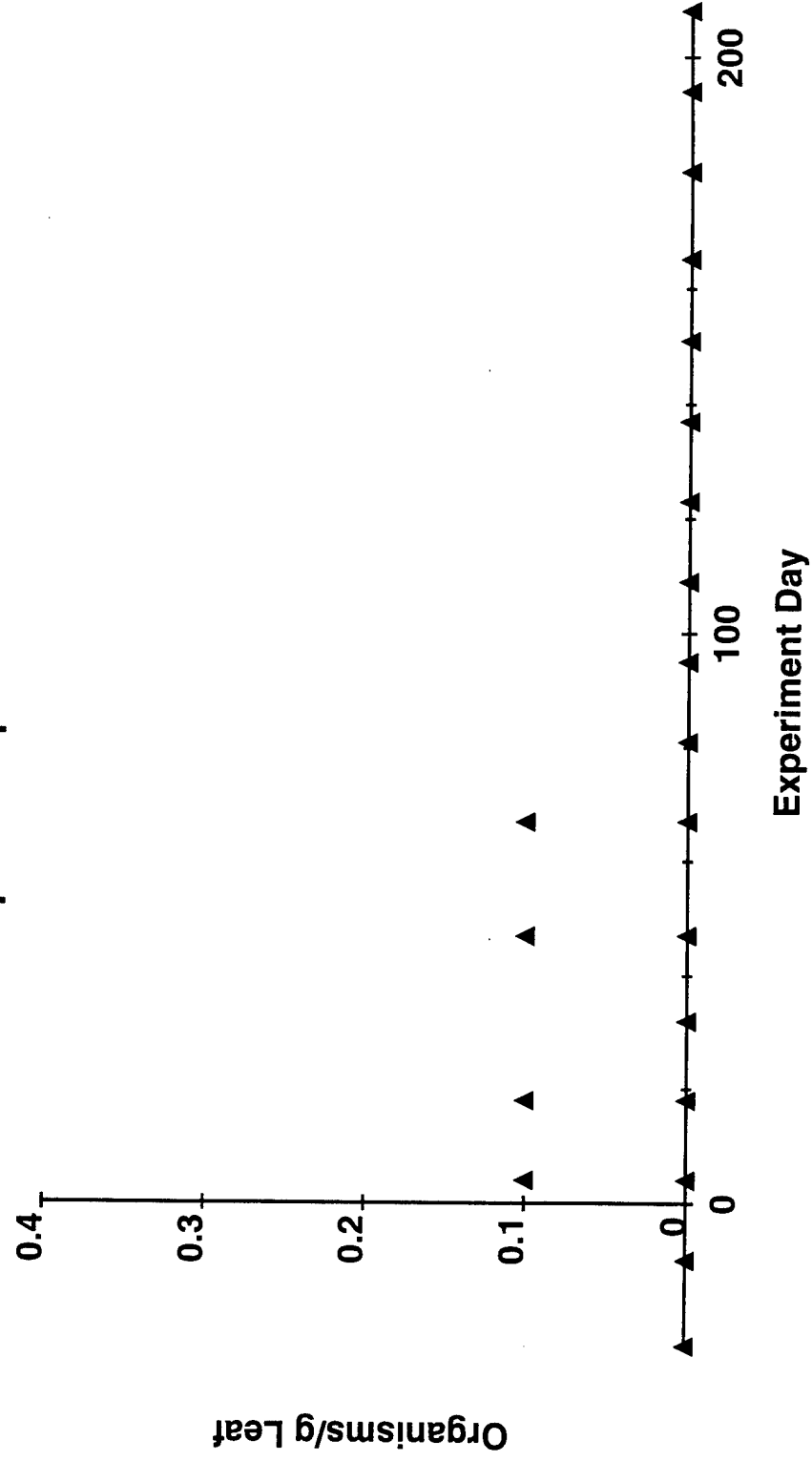
Figure 38a. Insects
Hemiptera - High Dose
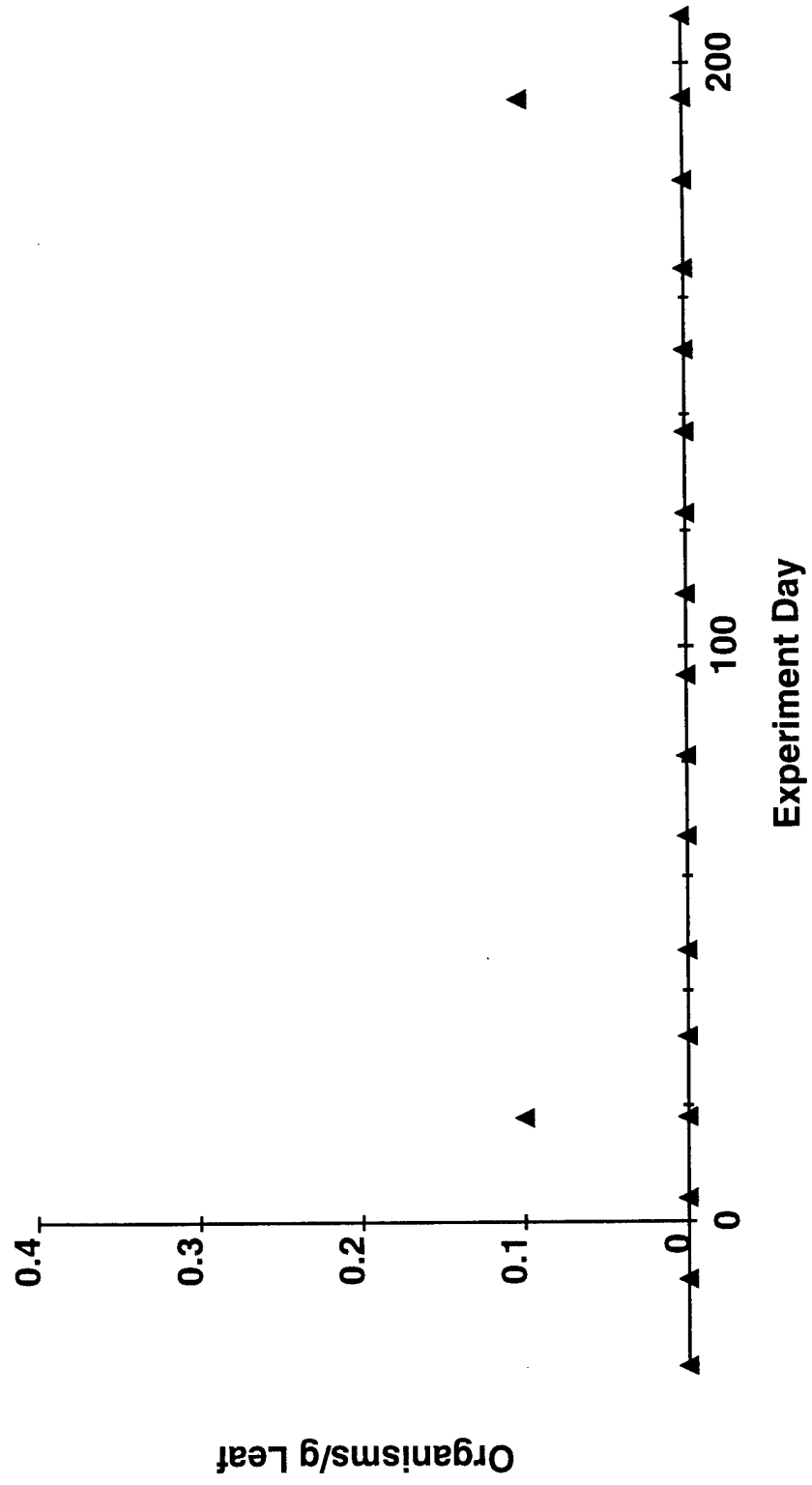
Figure 38b. Insects
Hemiptera - Mid-Dose
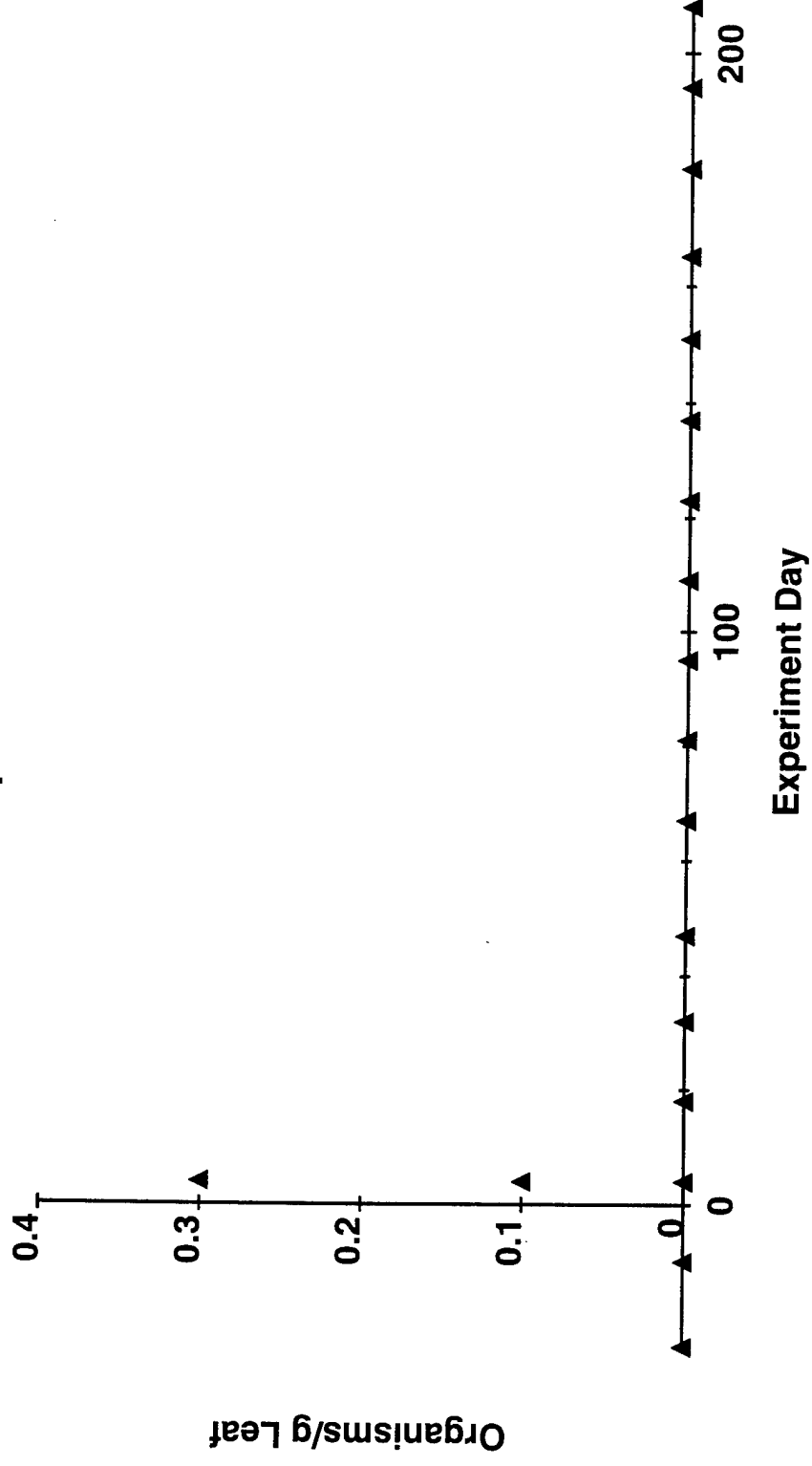
Figure 38c. Insects
Hemiptera - Low Dose

Organisms/g Leaf

Experiment Day
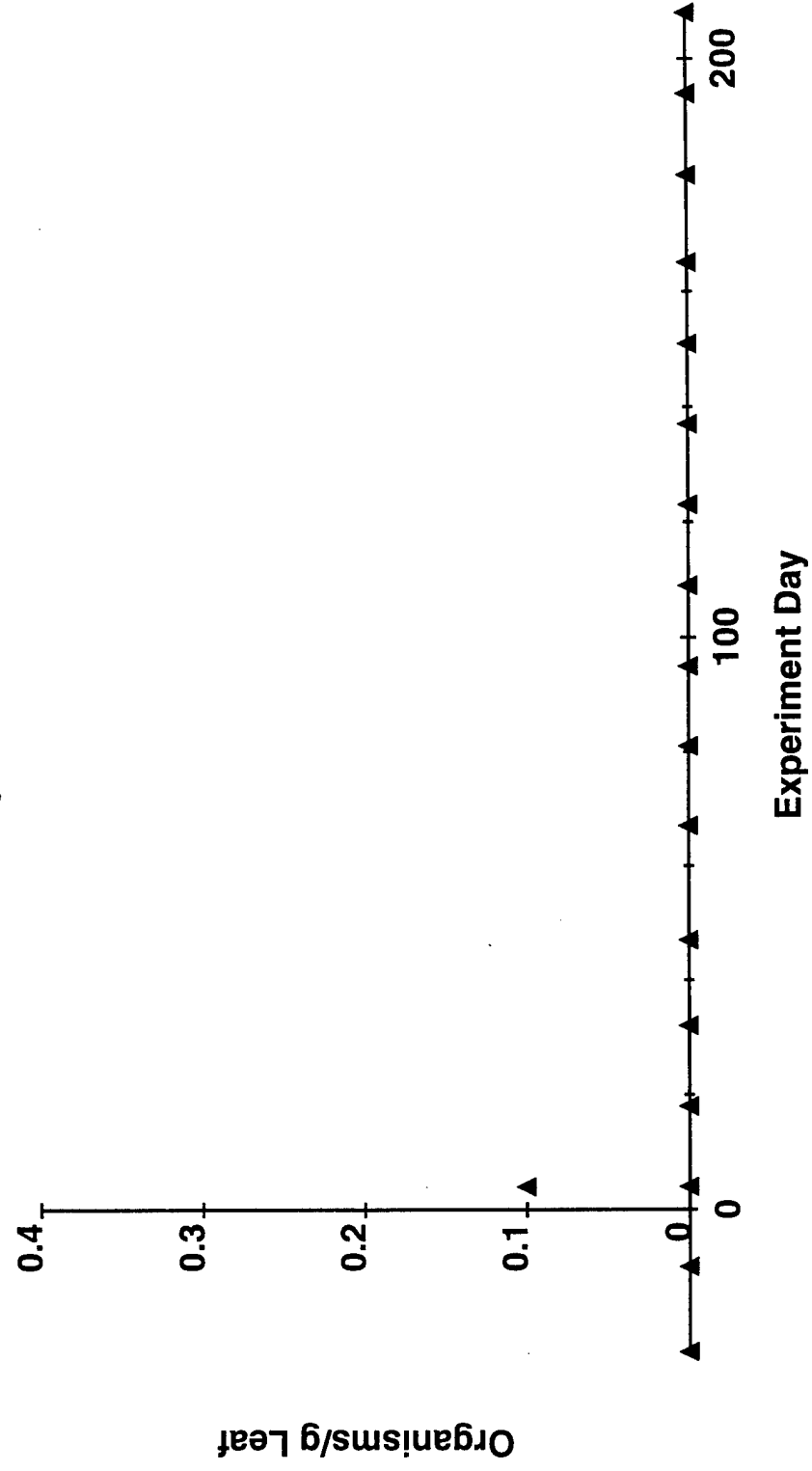
Figure 38d. Insects
Hemiptera - Undosed
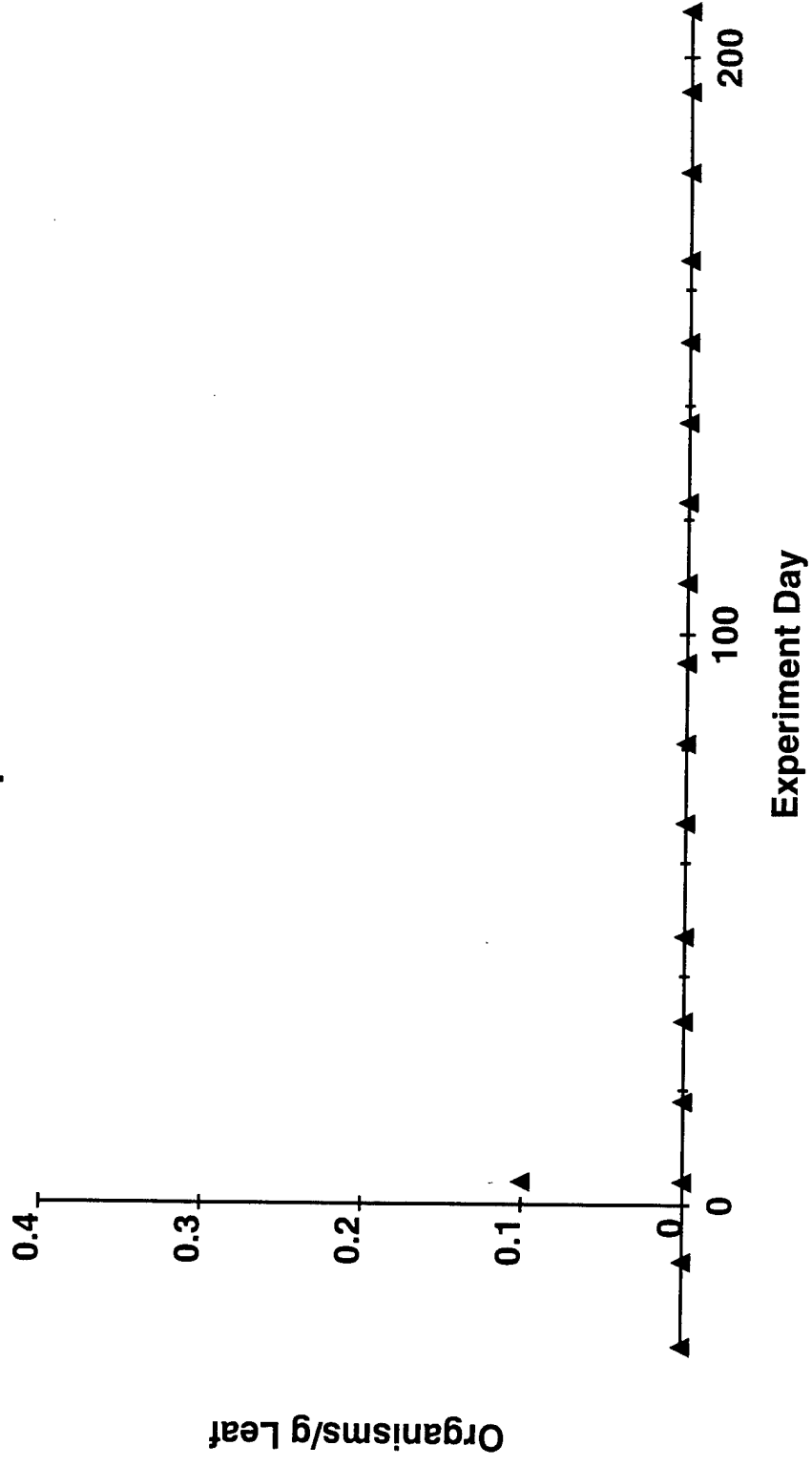
Figure 39a. Insects
Odonata, Damselflies - High Dose
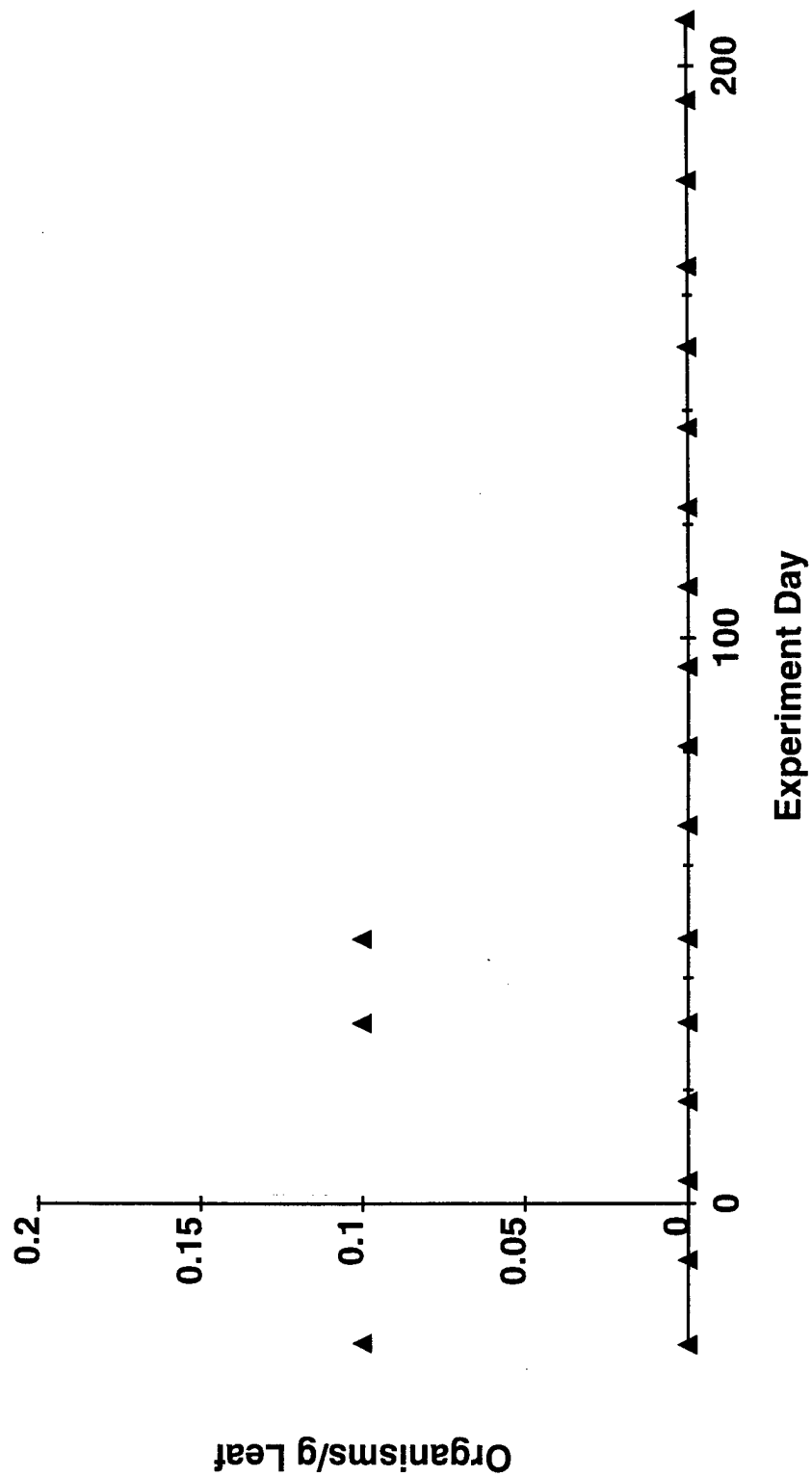
Figure 39b. Insects
Odonata, Damselflies - Mid-Dose
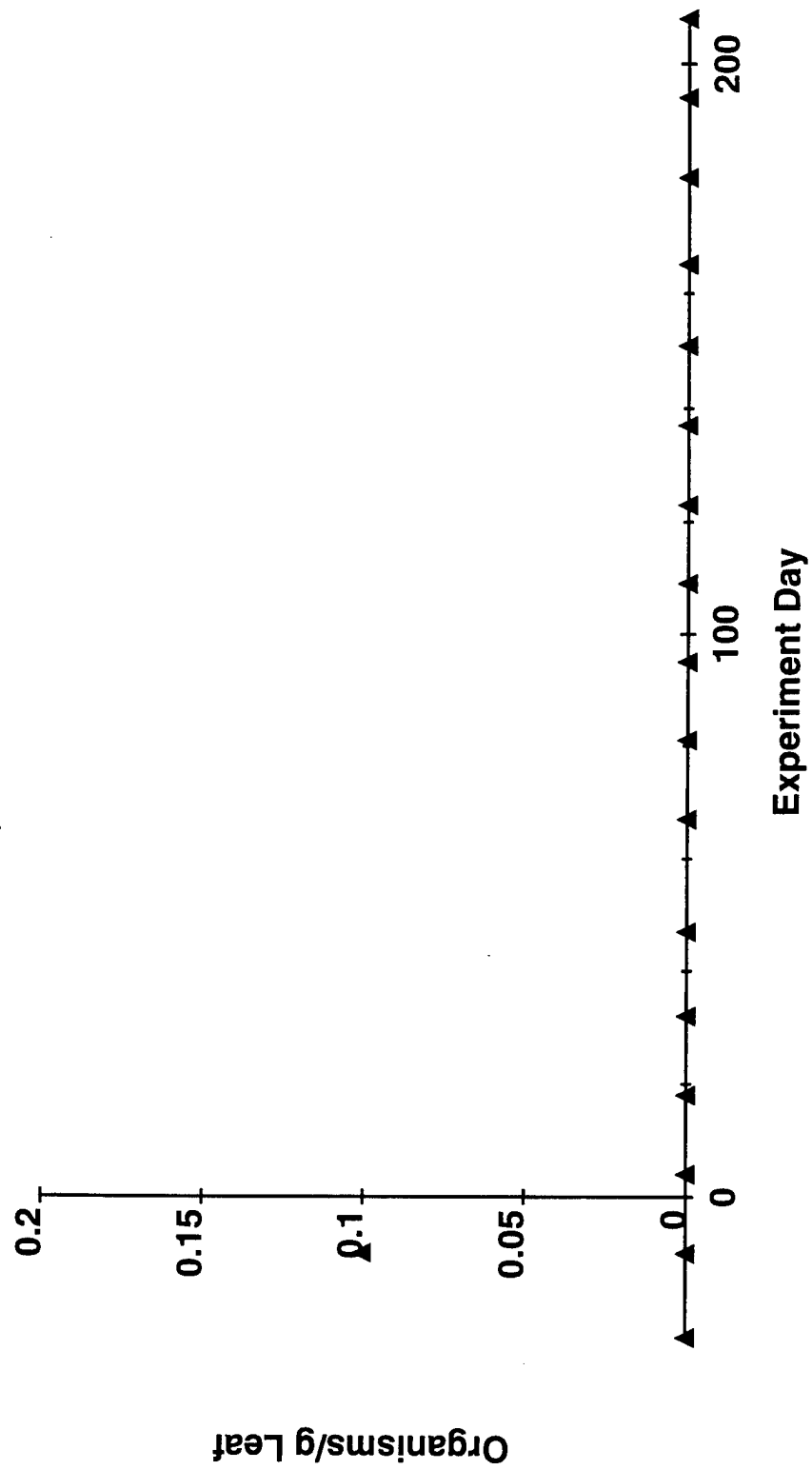
Figure 39c. Insects
Odonata, Damselflies - Low Dose
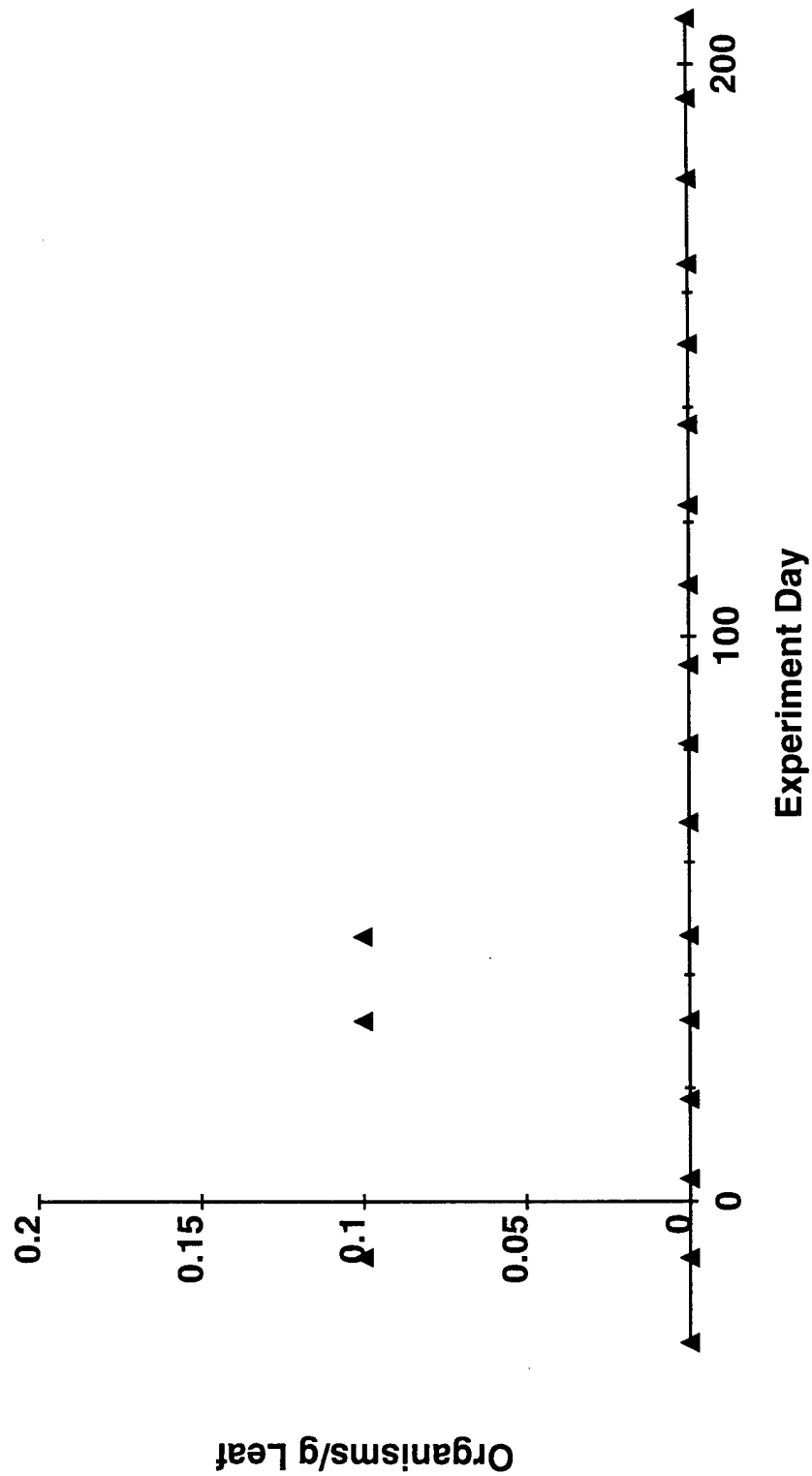
Figure 39d. Insects
Odonata, Damselflies - Undosed
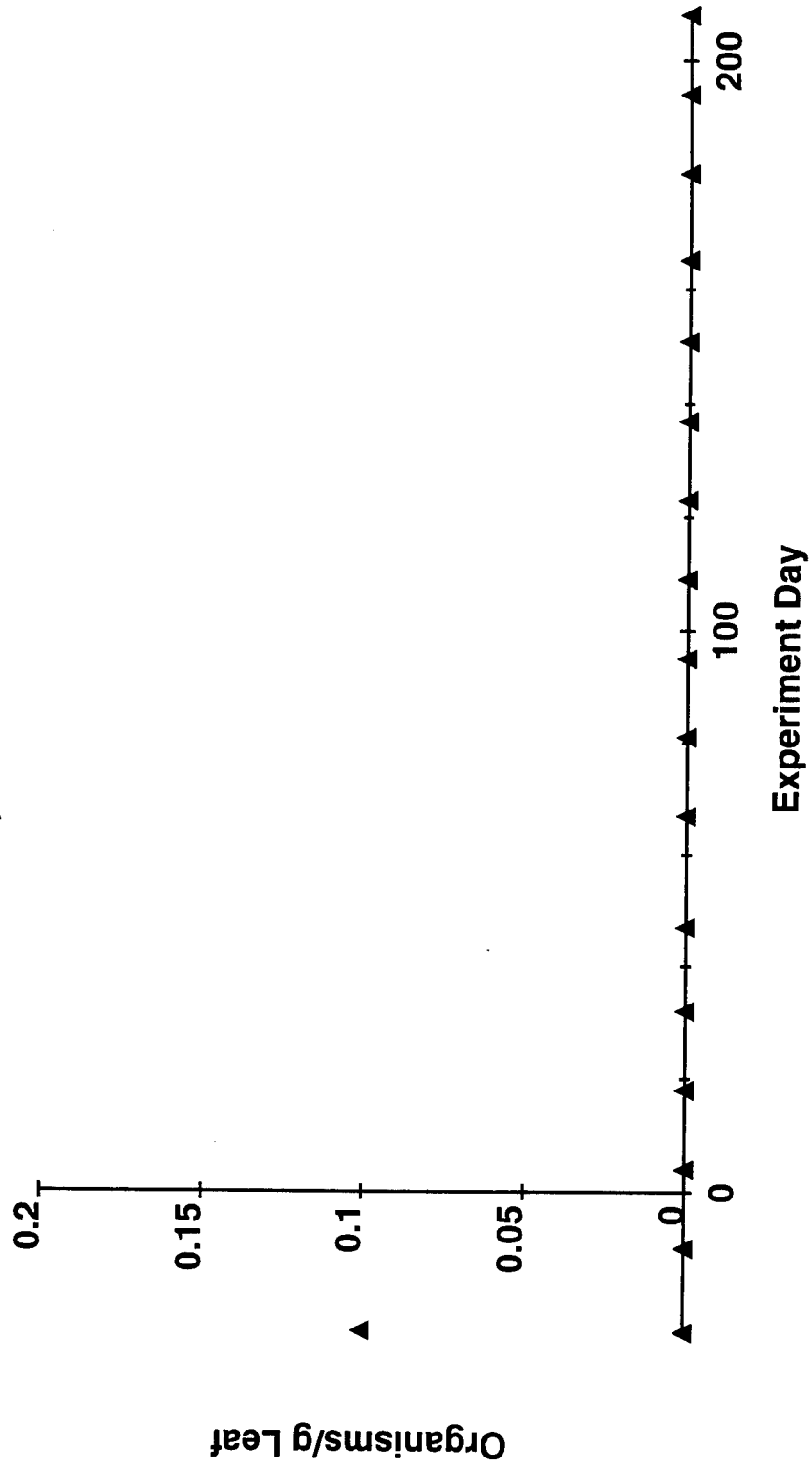
Figure 40a. Insects
Odonata, Dragonflies - High Dose
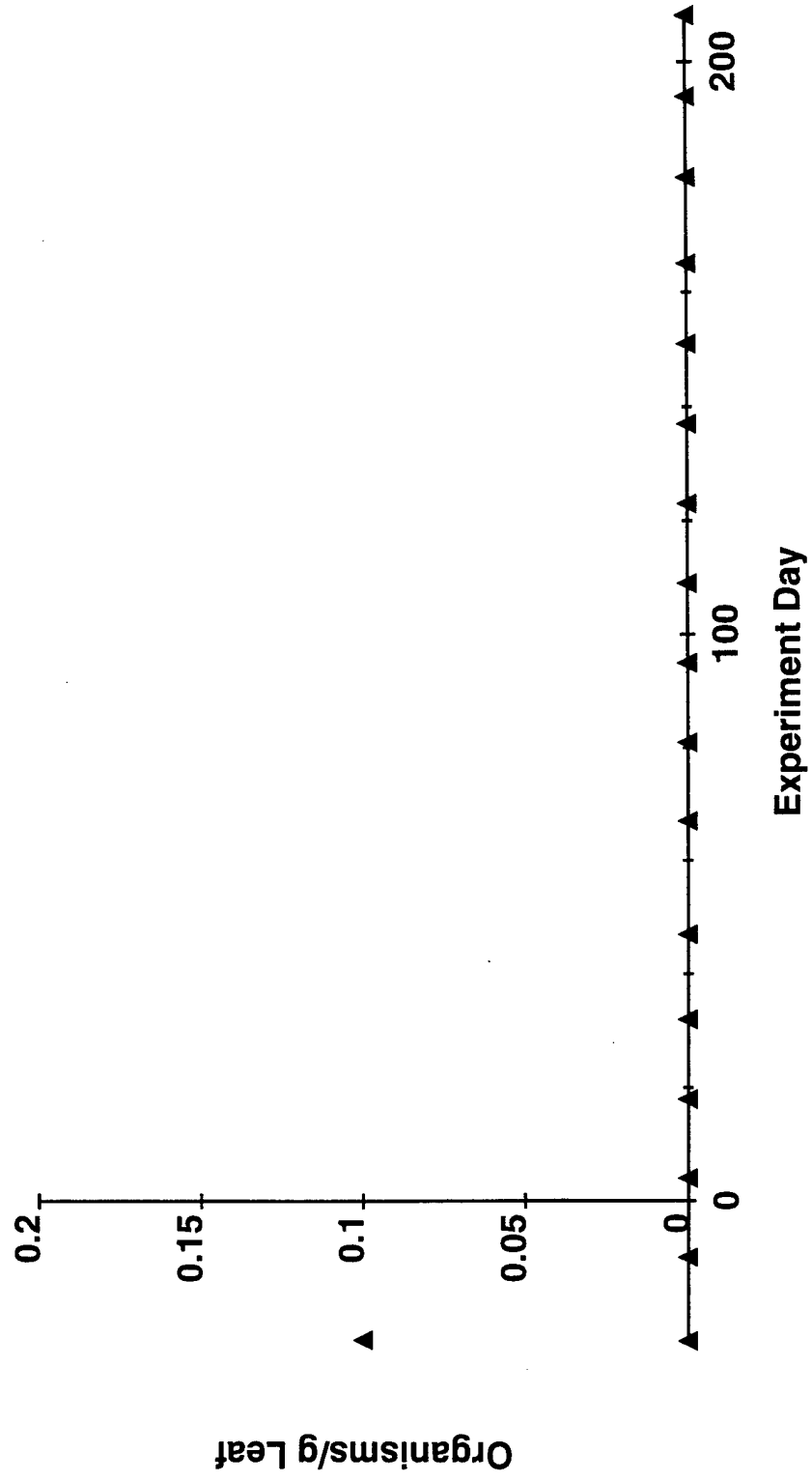
Figure 40b. Insects
Odonata, Dragonflies - Mid-Dose
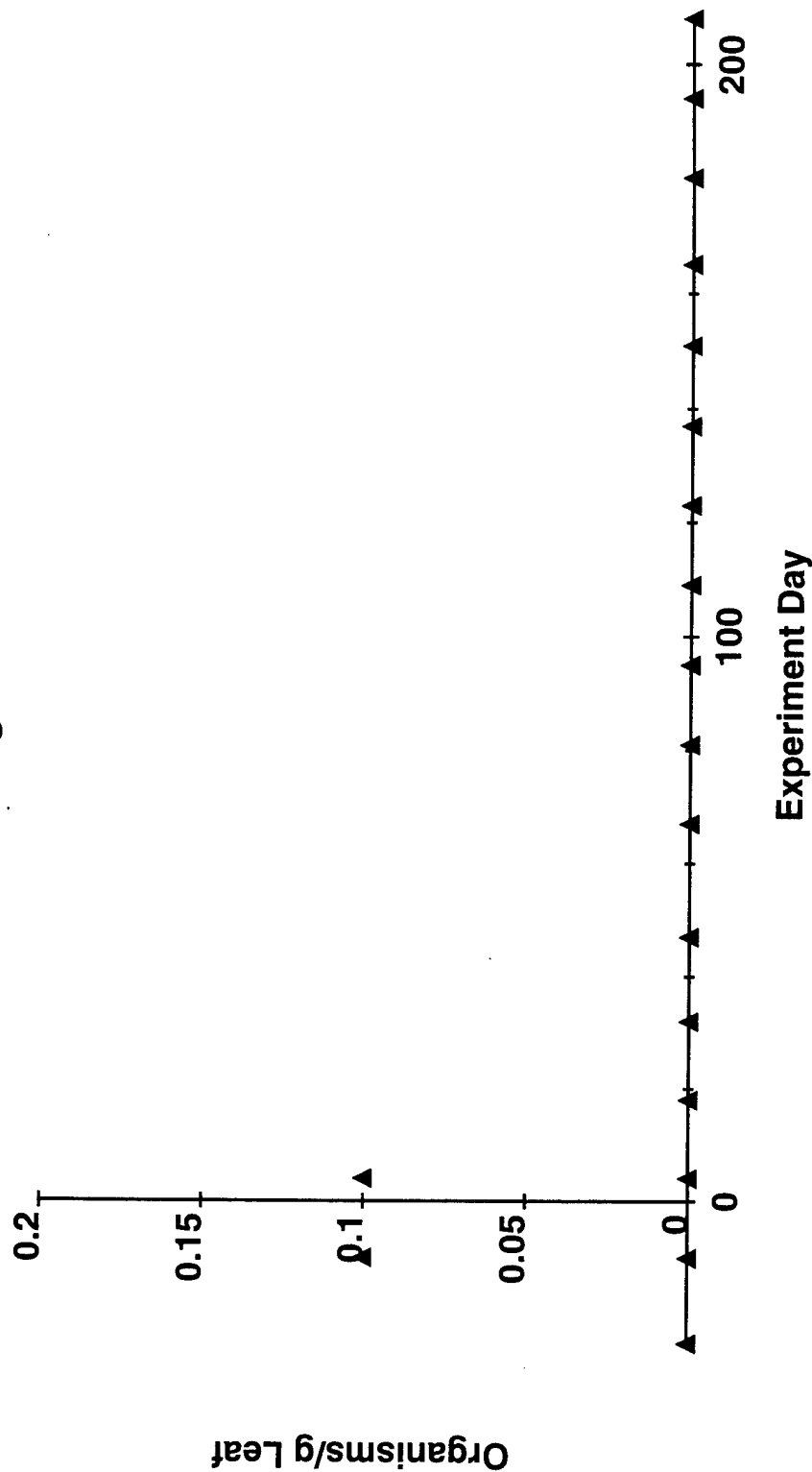
Figure 40c. Insects
Odonata, Dragonflies - Low Dose
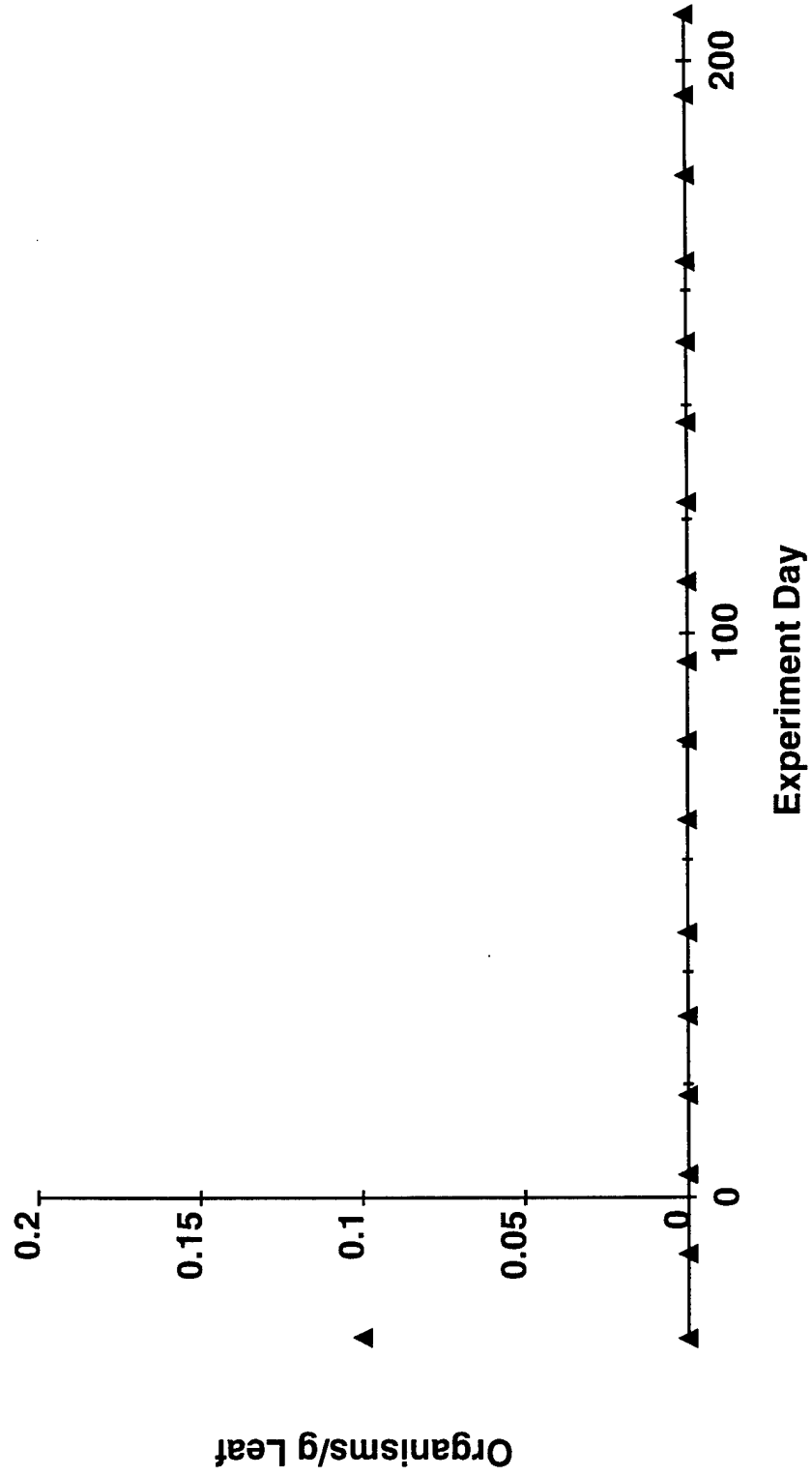
Figure 40d. Insects
Odonata, Dragonflies - Undosed
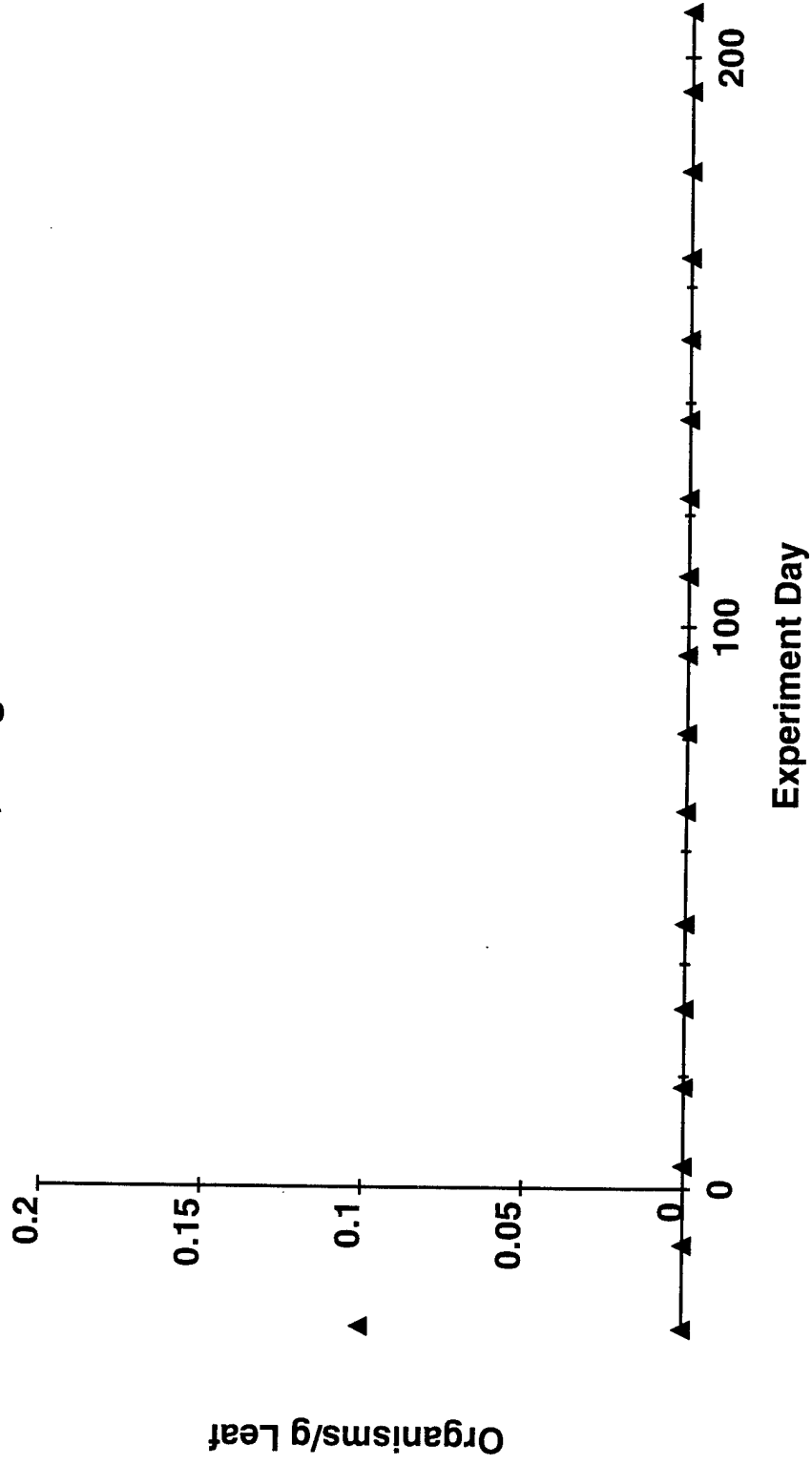
Figure 41a. Insects
Plecoptera - High Dose
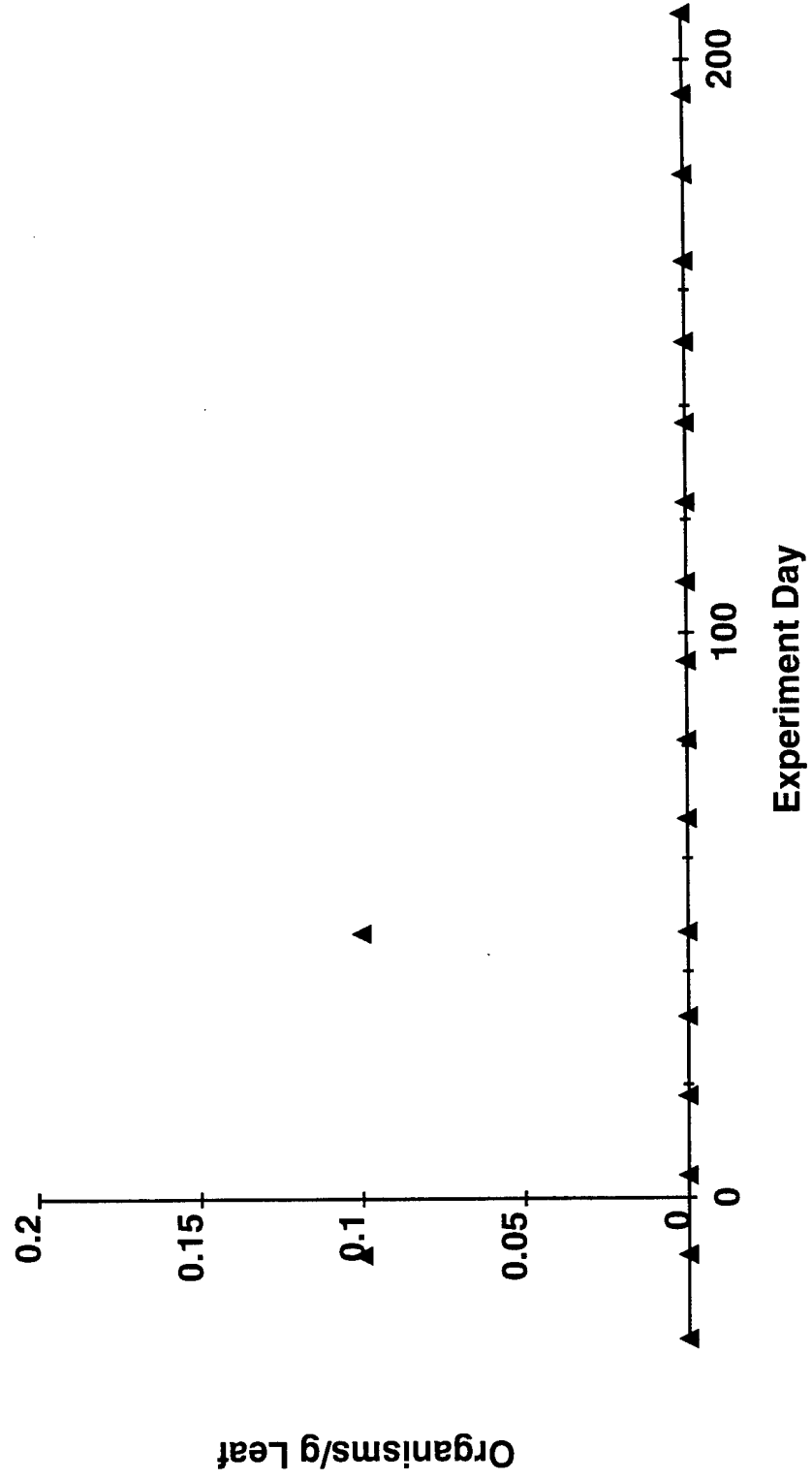
Figure 41b. Insects
Plecoptera - Mid-Dose

Organisms/g Leaf

Experiment Day
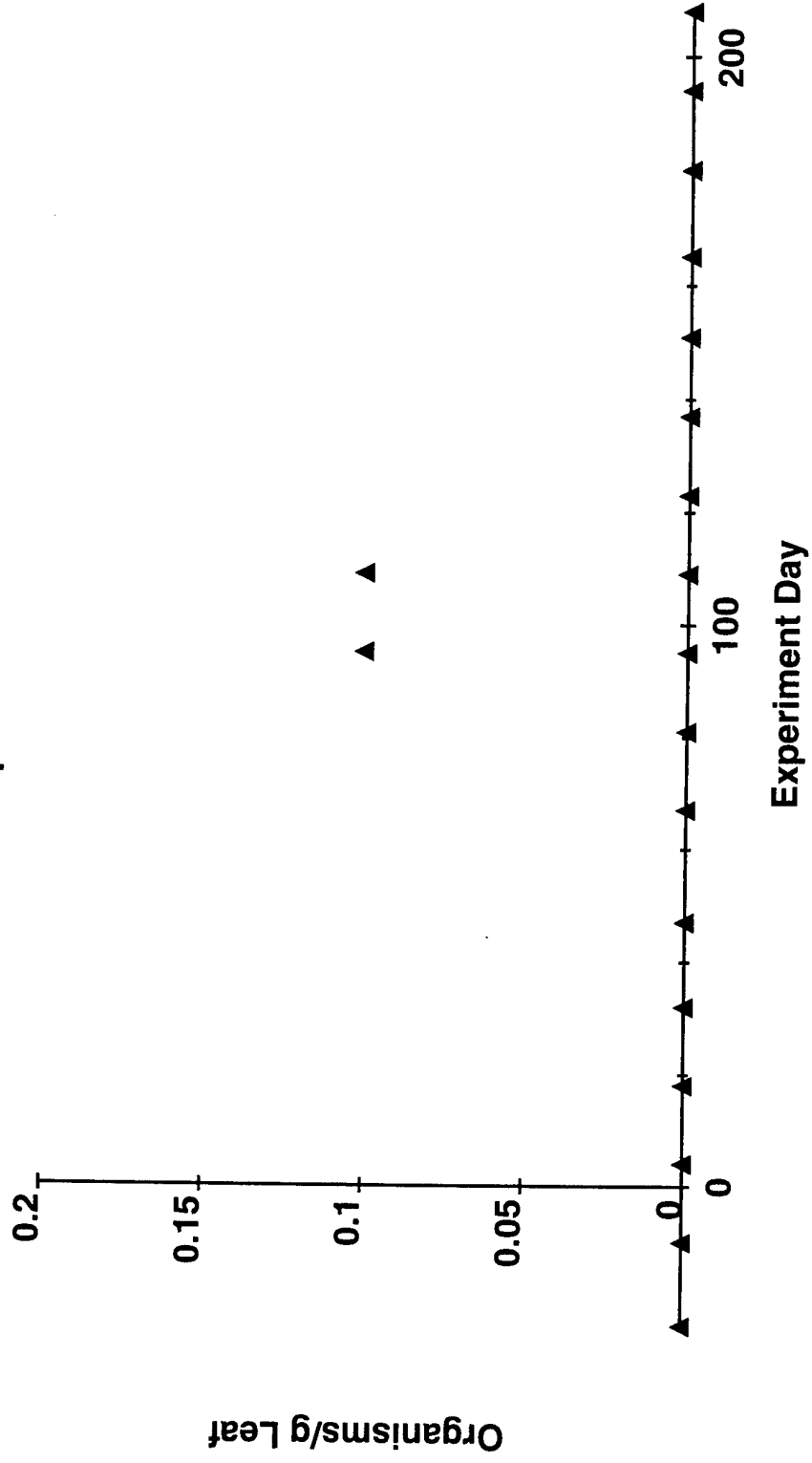
Figure 41c. Insects
Plecoptera - Low Dose

Organisms/g Leaf

Experiment Day
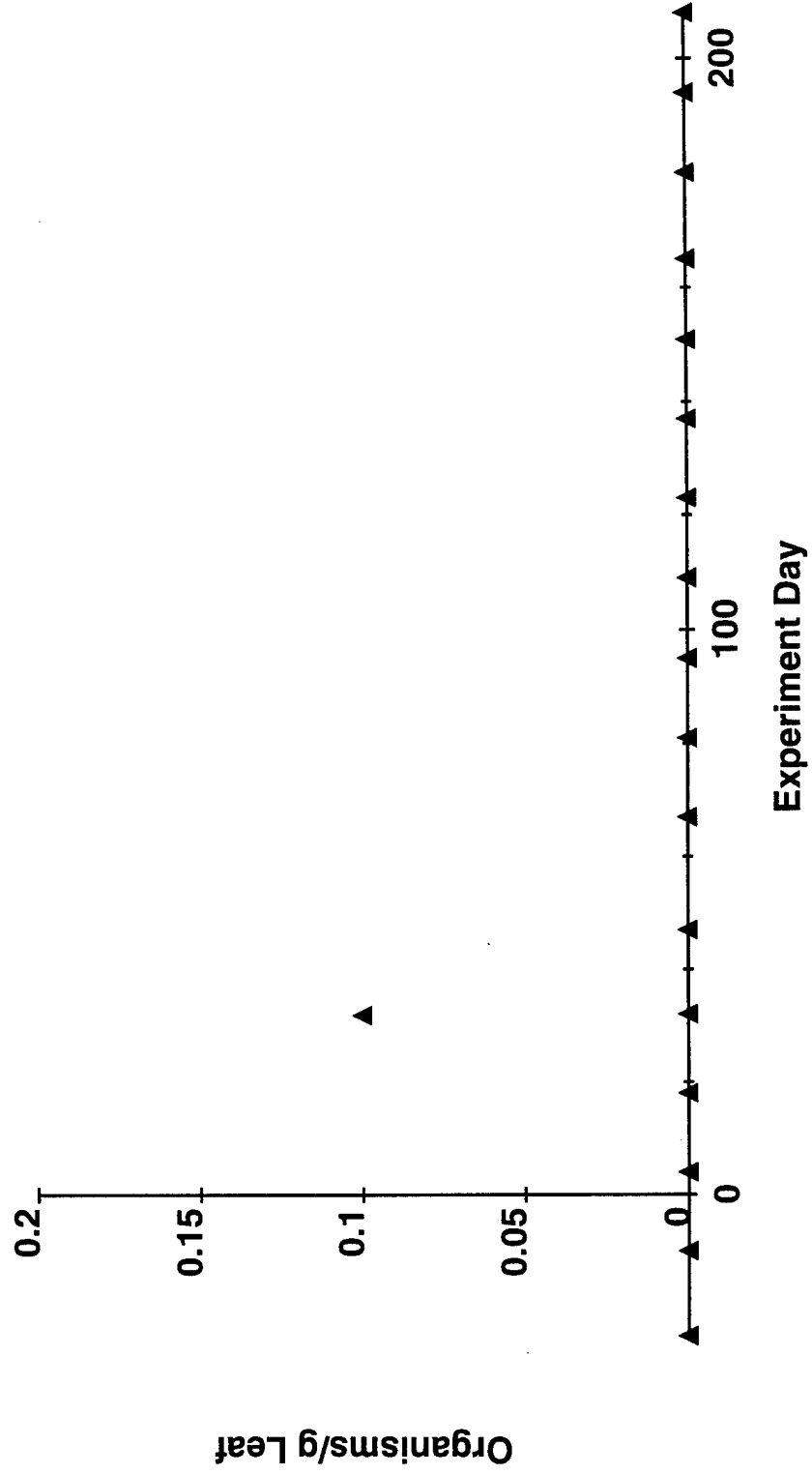
Figure 41d. Insects
Plecoptera - Undosed
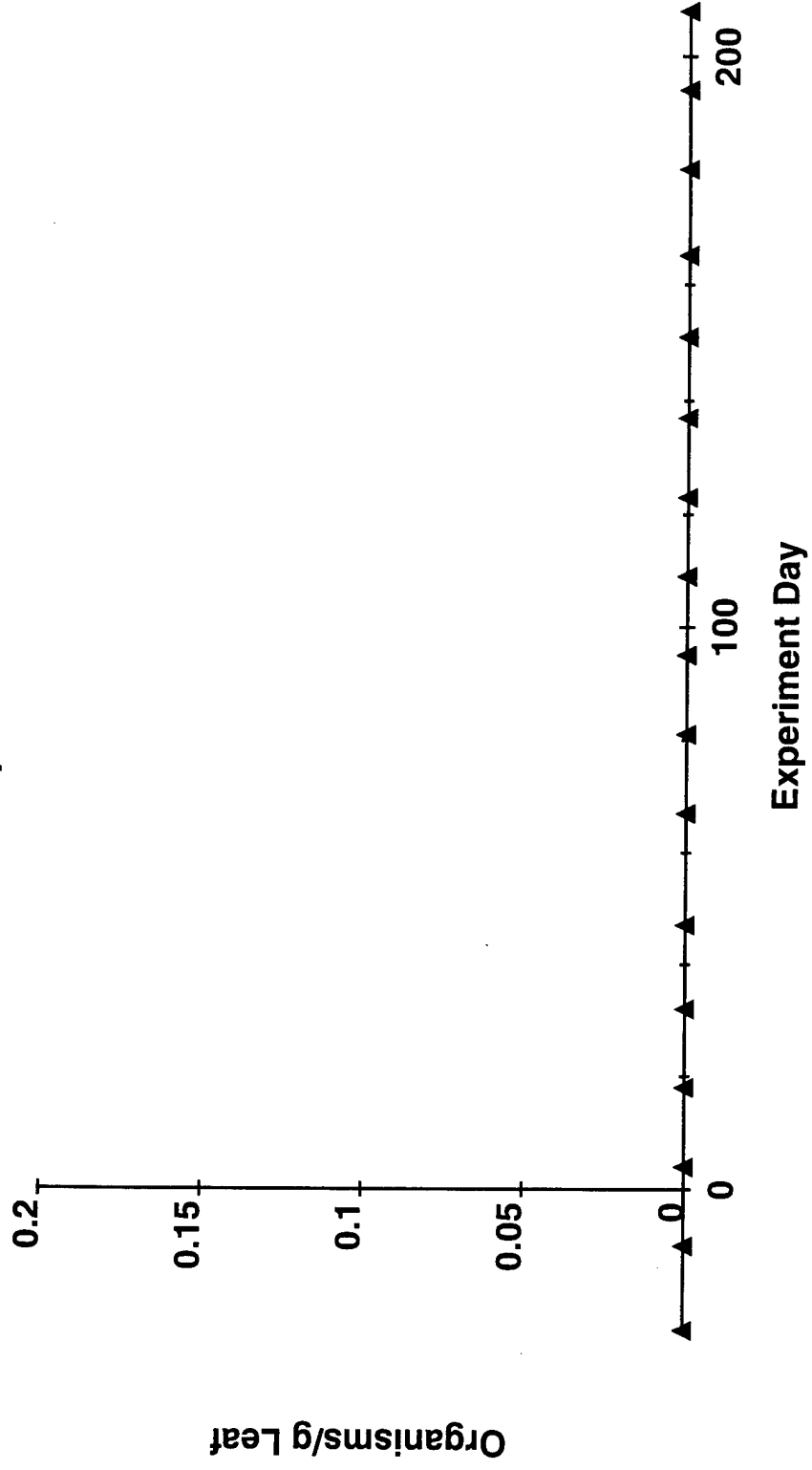
Figure 42a. Insects
Trichoptera - High Dose
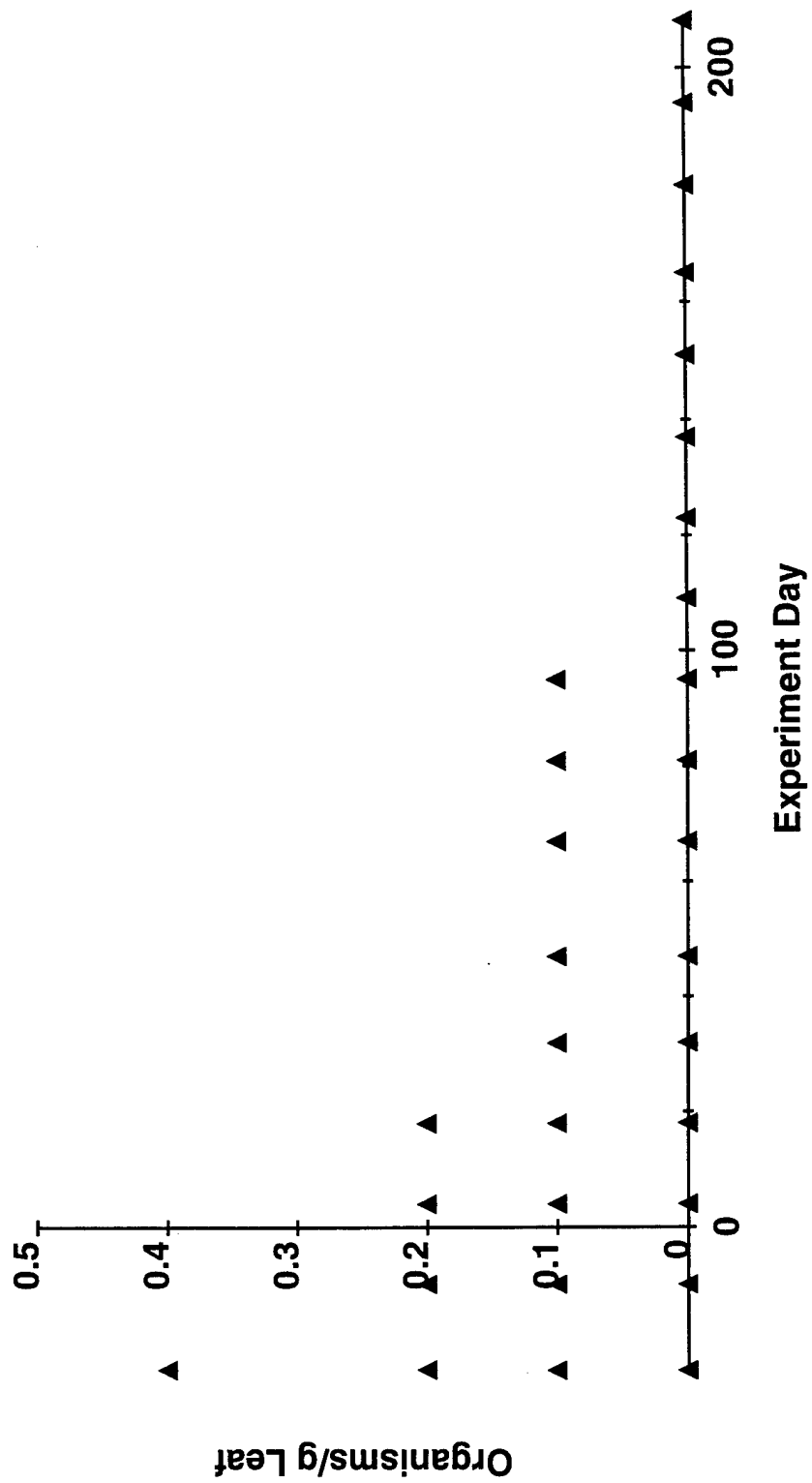
Figure 42b. Insects
Trichoptera- Mid-Dose
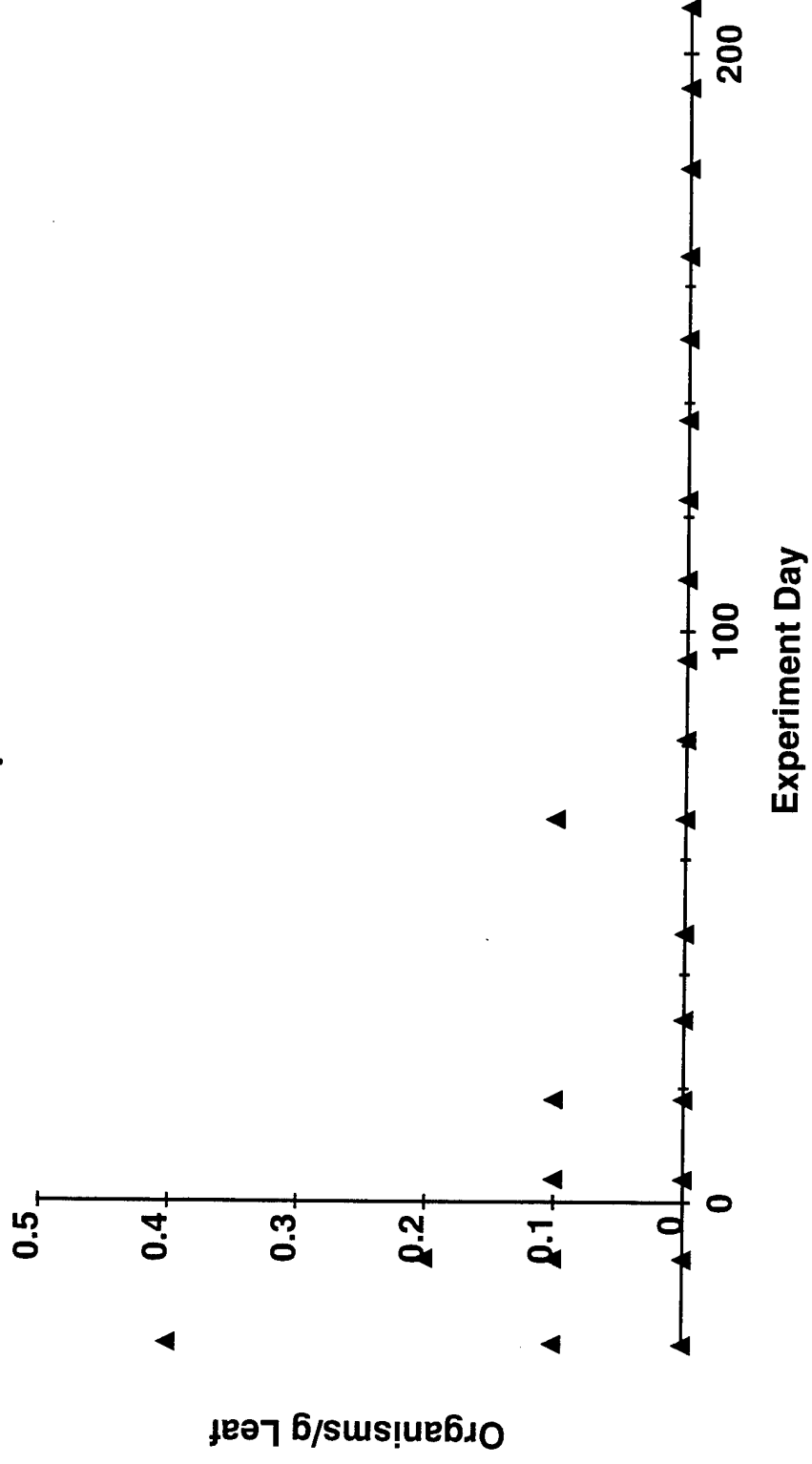
Figure 42c. Insects
Trichoptera - Low Dose
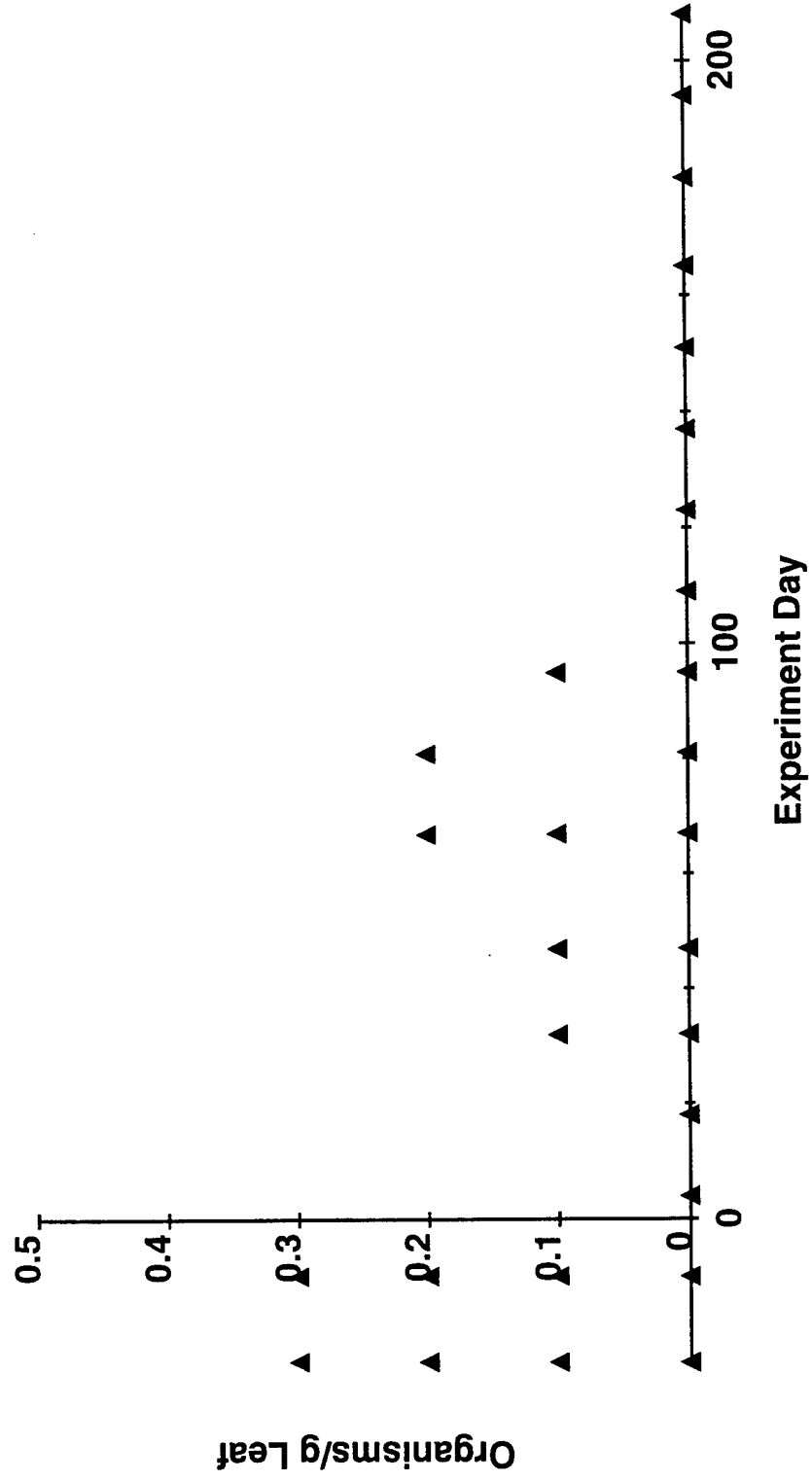
Figure 42d. Insects
Trichoptera - Undosed
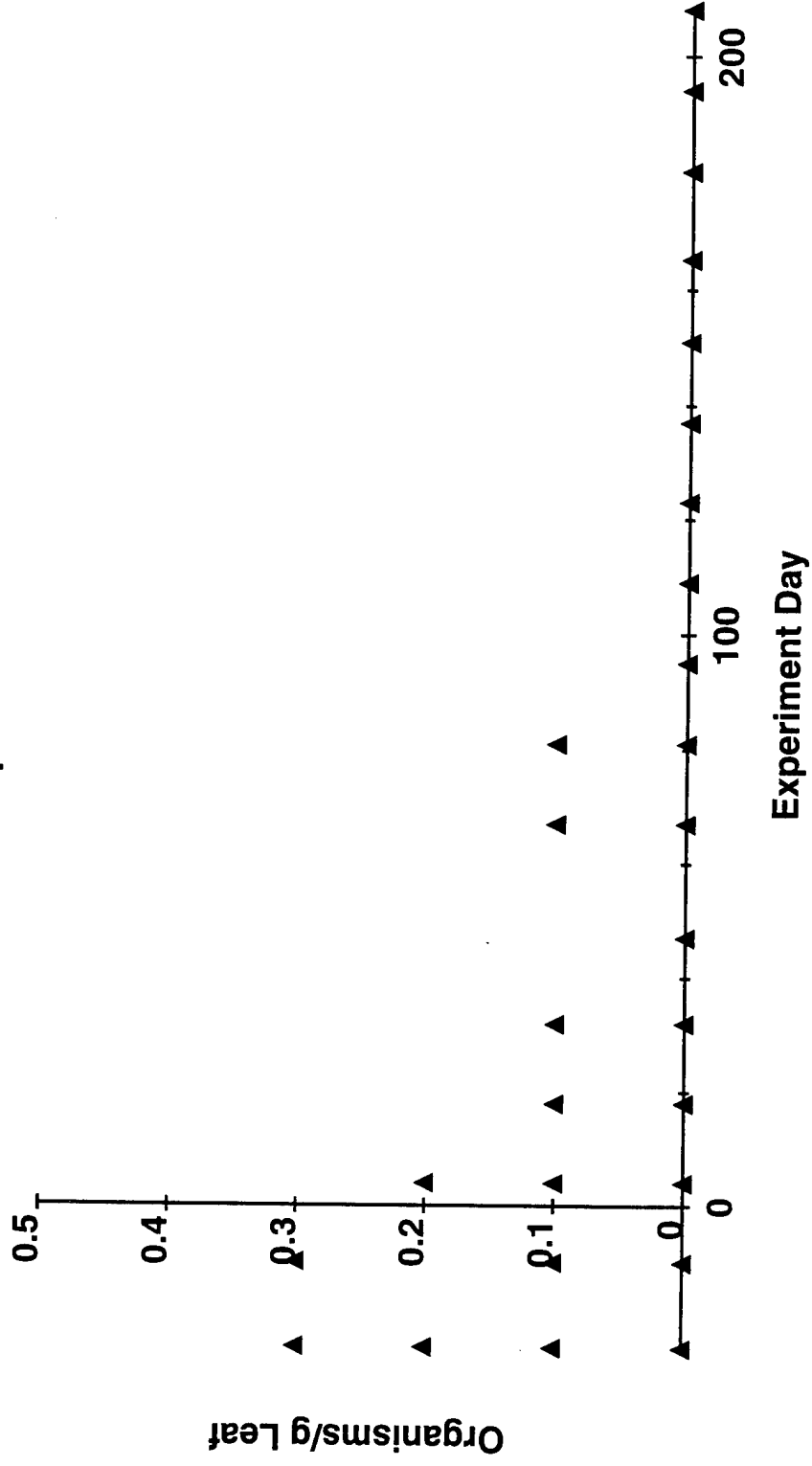
Figure 43a. Molluscs
Clams - High Dose

Organisms/g Leaf

Experiment Day
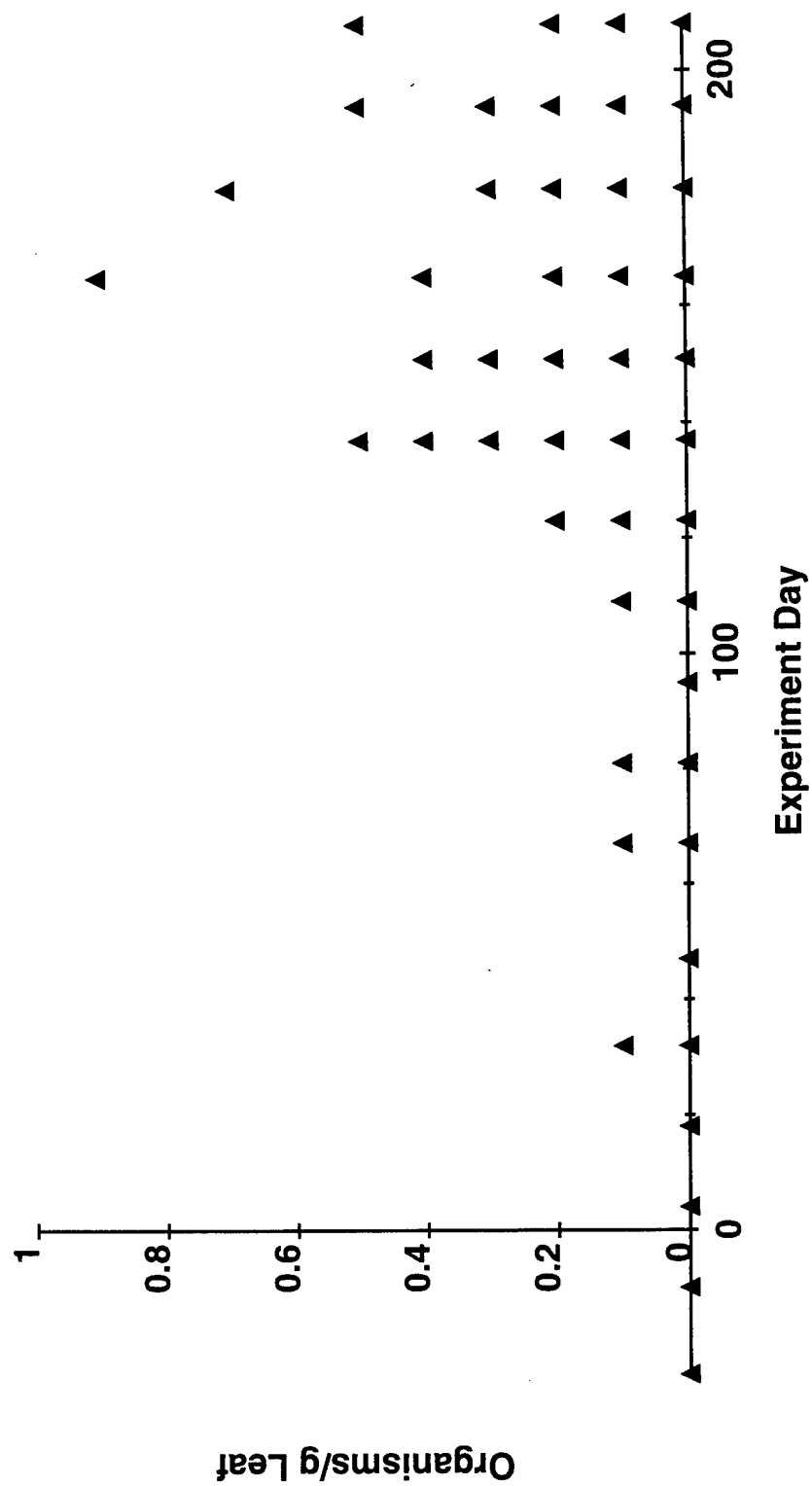
Figure 43b. Molluscs
Clams - Mid-Dose

Organisms/g Leaf

Experiment Day
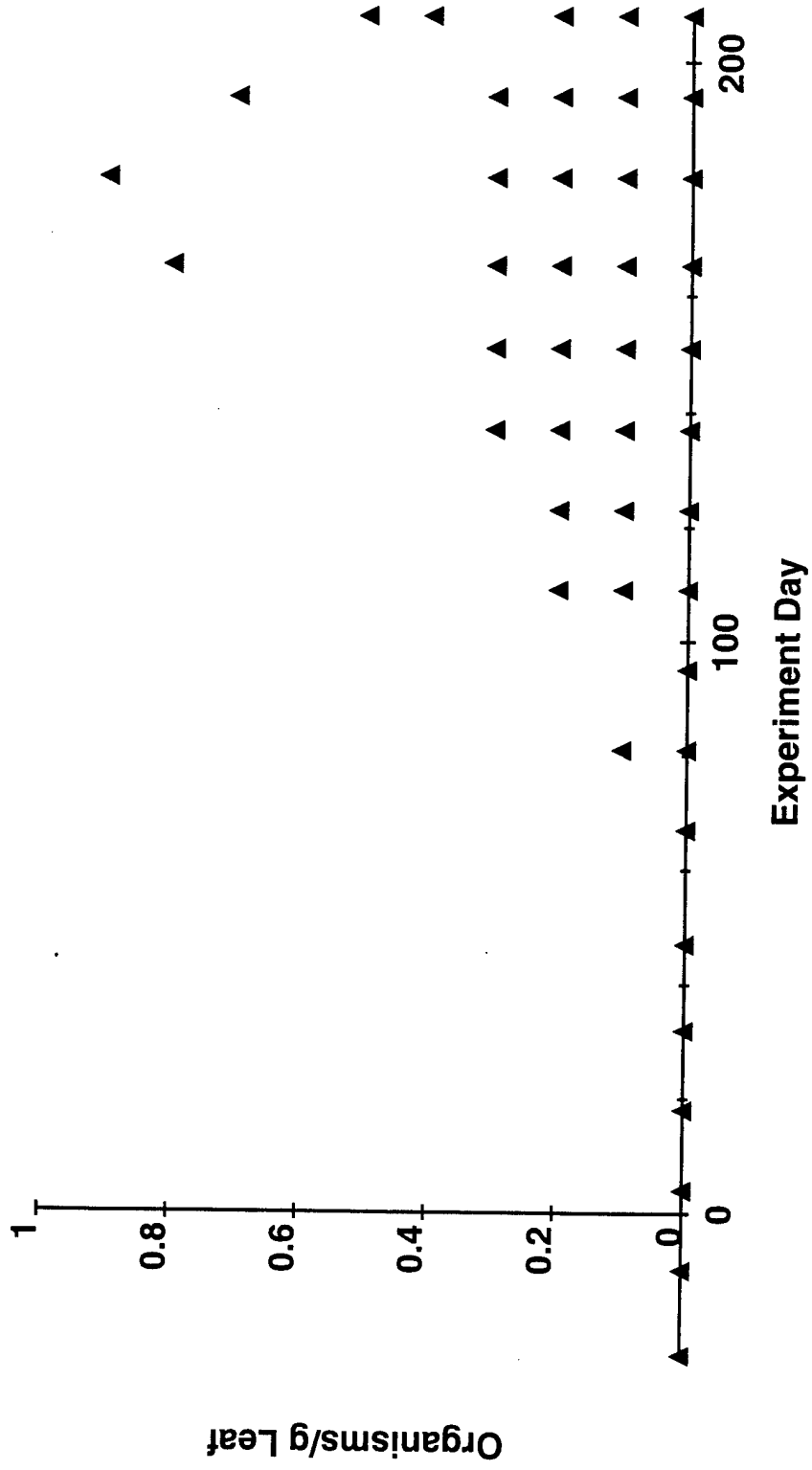
Figure 43c. Molluscs
Clams - Low Dose

Organisms/g Leaf

Experiment Day
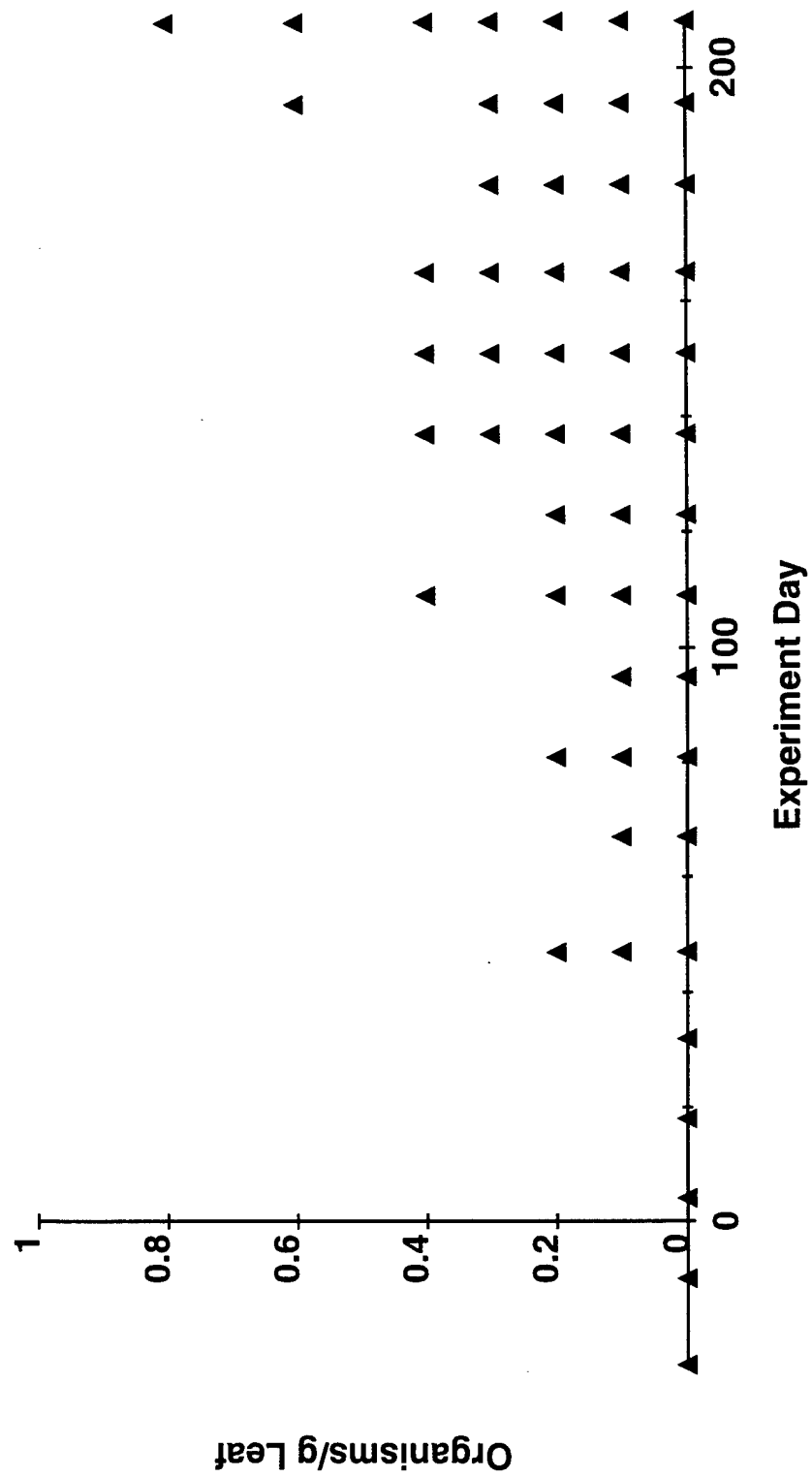
Figure 43d. Molluscs
Clams - Undosed
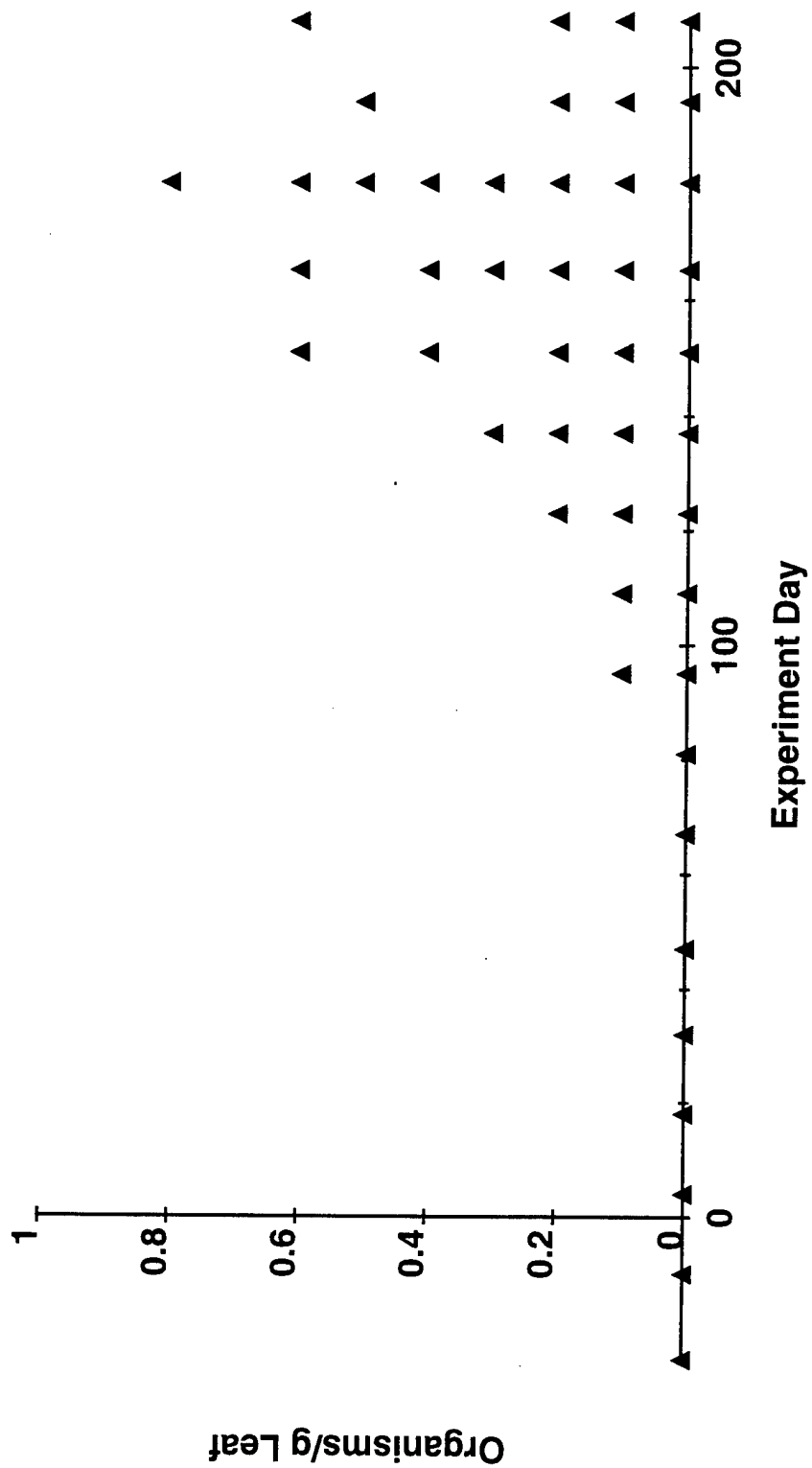
Figure 44a. Molluscs
Limpets - High Dose
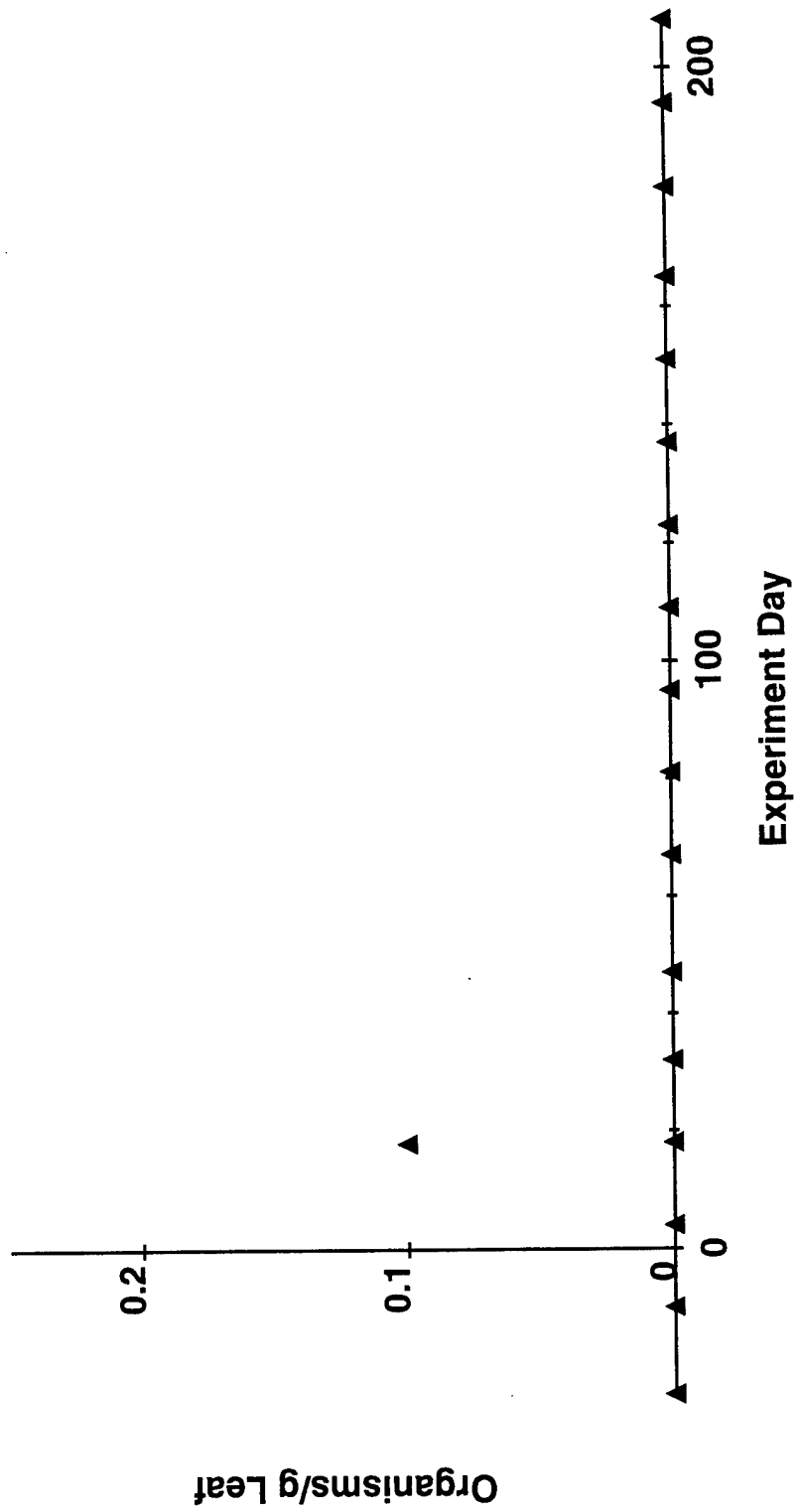
Figure 44b. Molluscs
Limpets - Mid-Dose

Organisms/g Leaf

Experiment Day

0   100   200

0.2

0.1

0
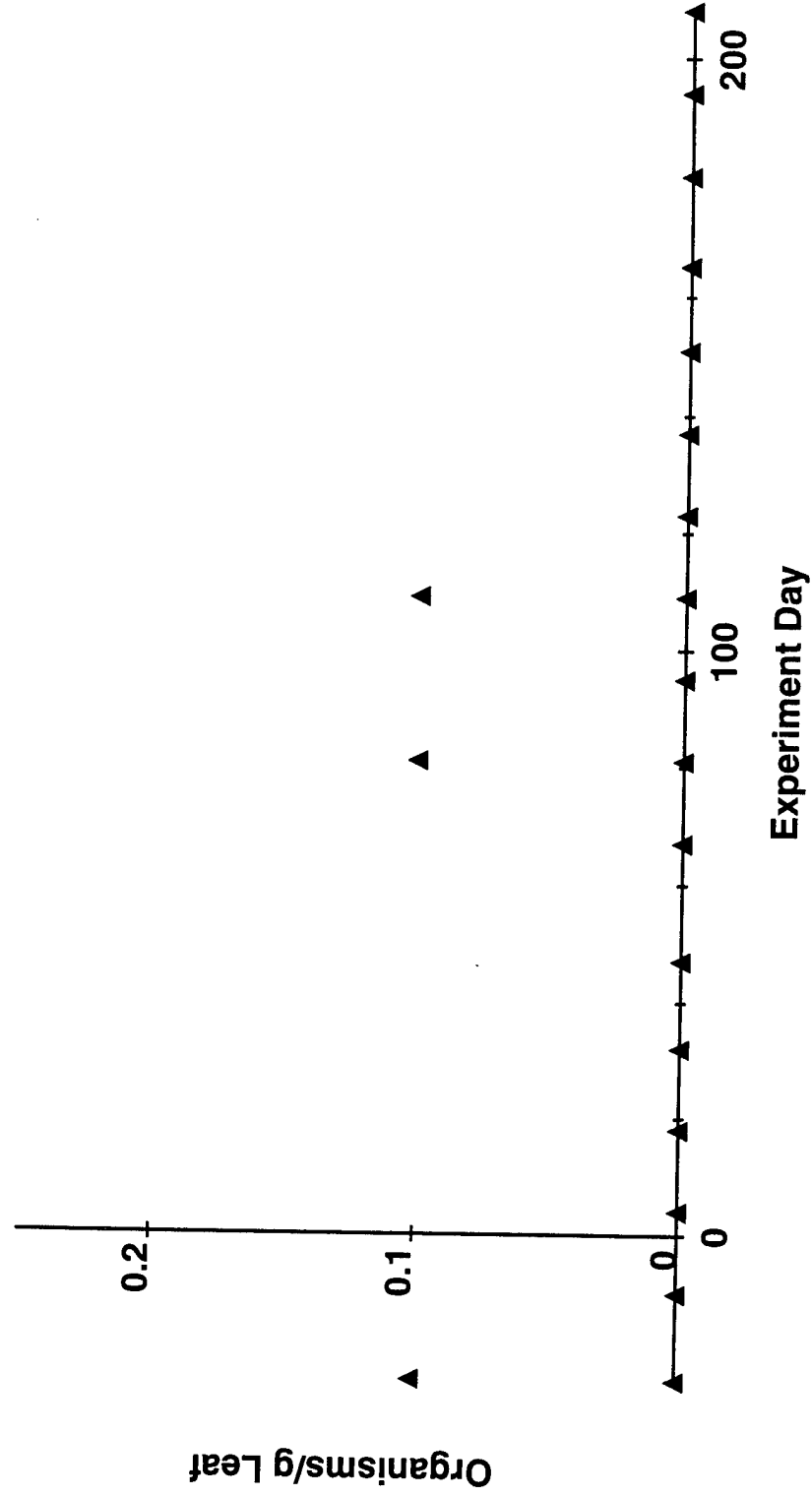
Figure 44c. Molluscs
Limpets - Low Dose
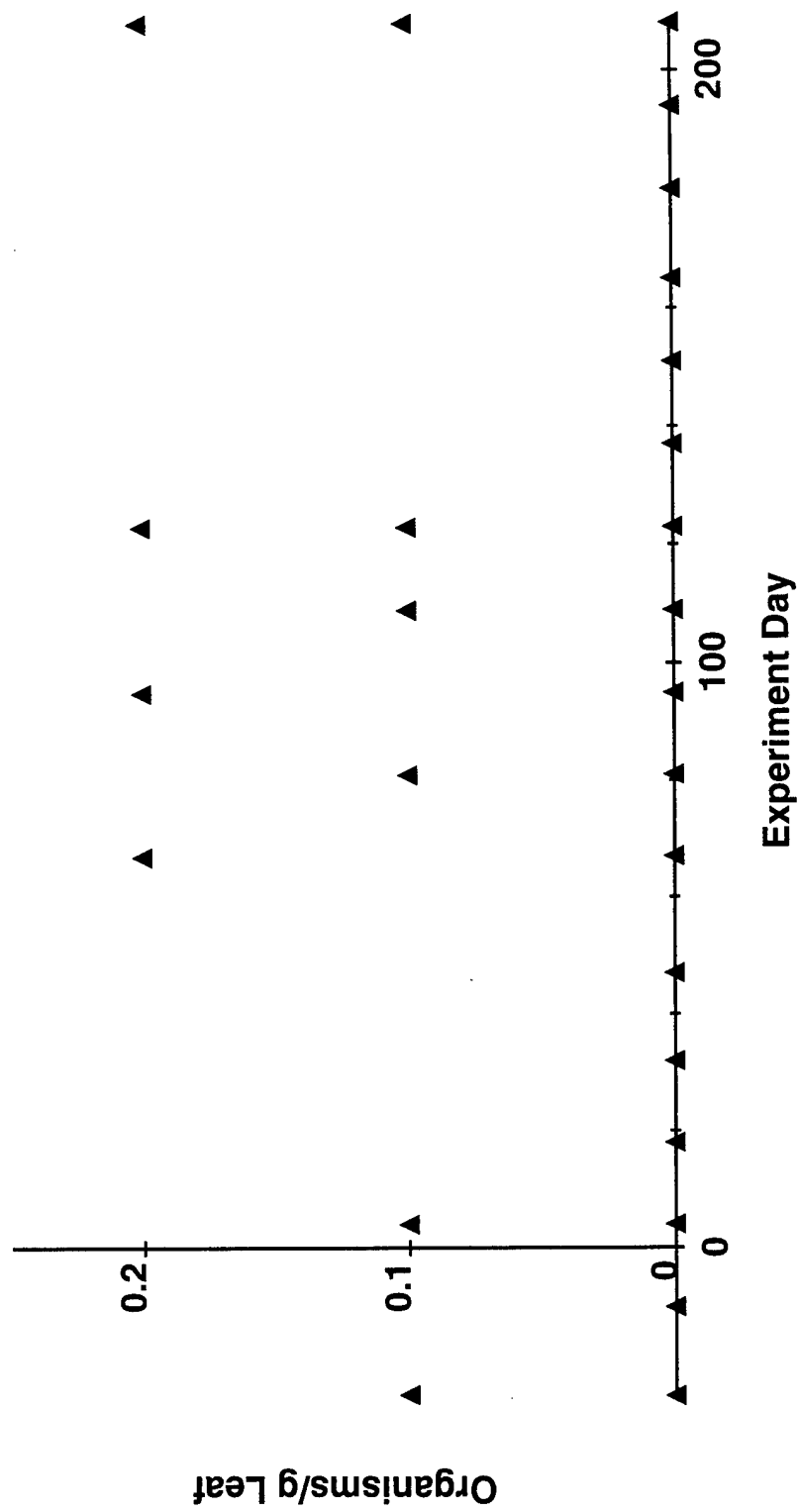
Figure 44d. Molluscs
Limpets - Undosed

Organisms/g Leaf

Experiment Day
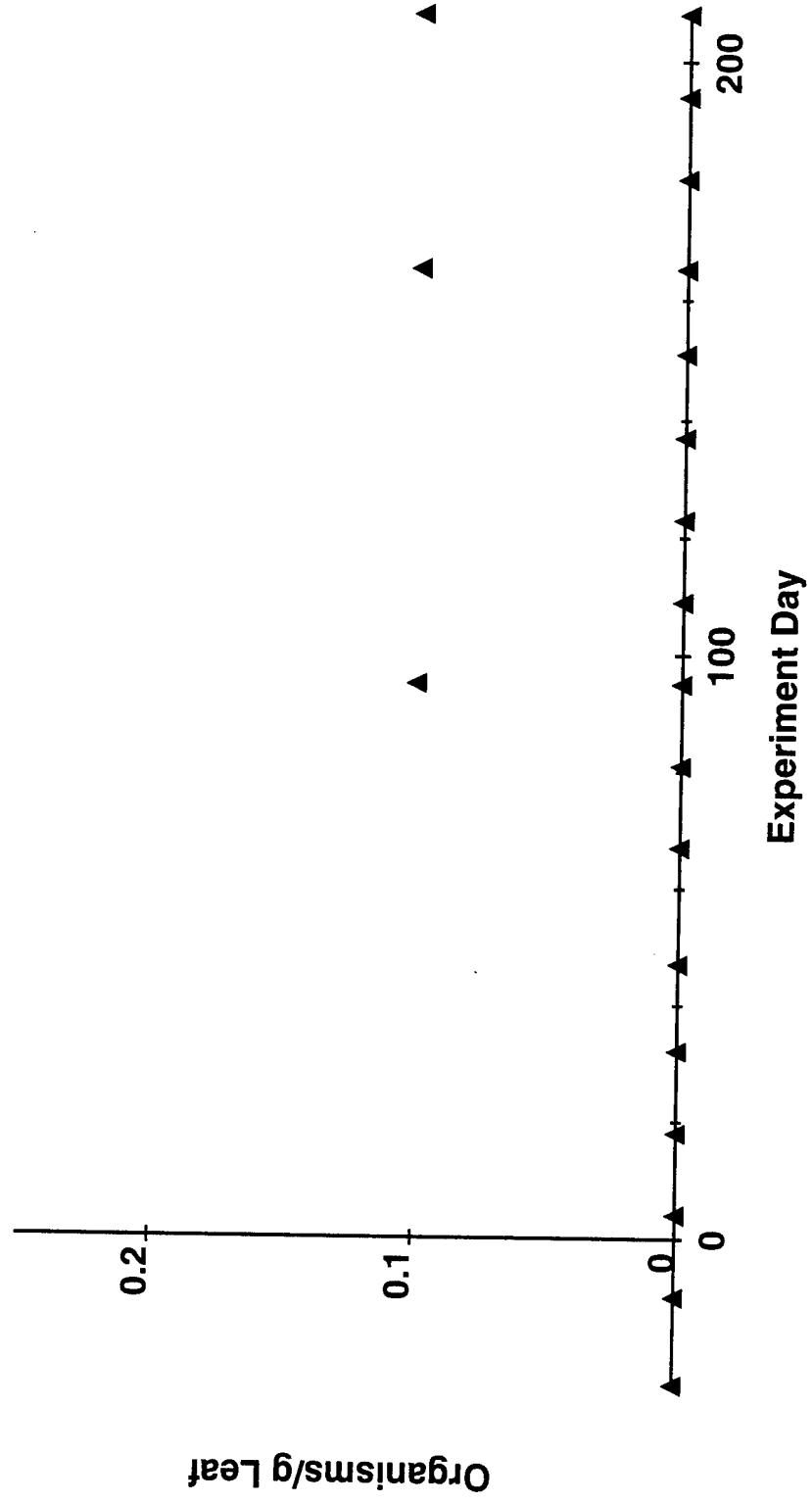
Figure 45a. Molluscs
Snails - High Dose
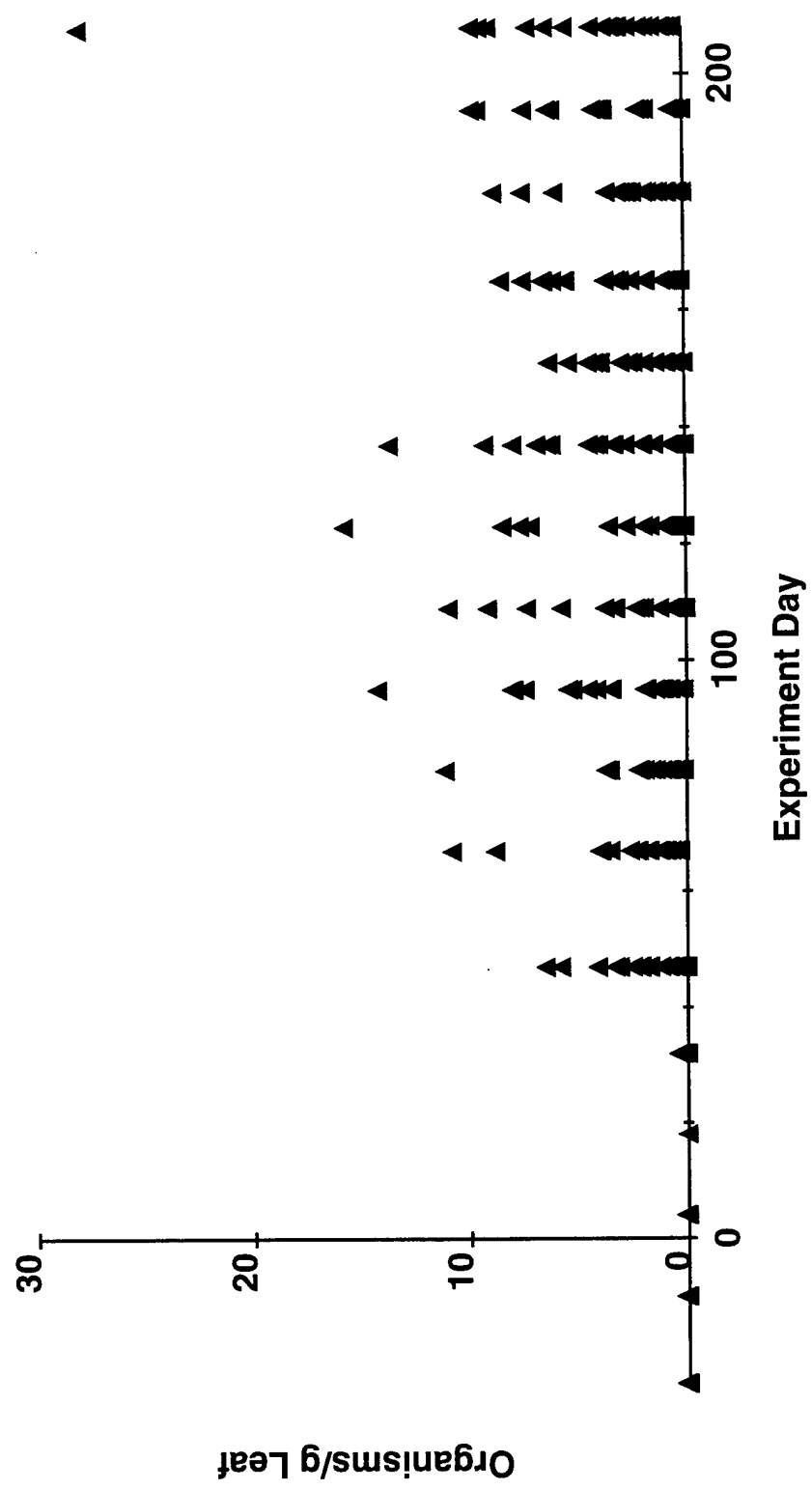
Figure 45b. Molluscs
Snails - Mid-Dose

Organisms/g Leaf

Experiment Day
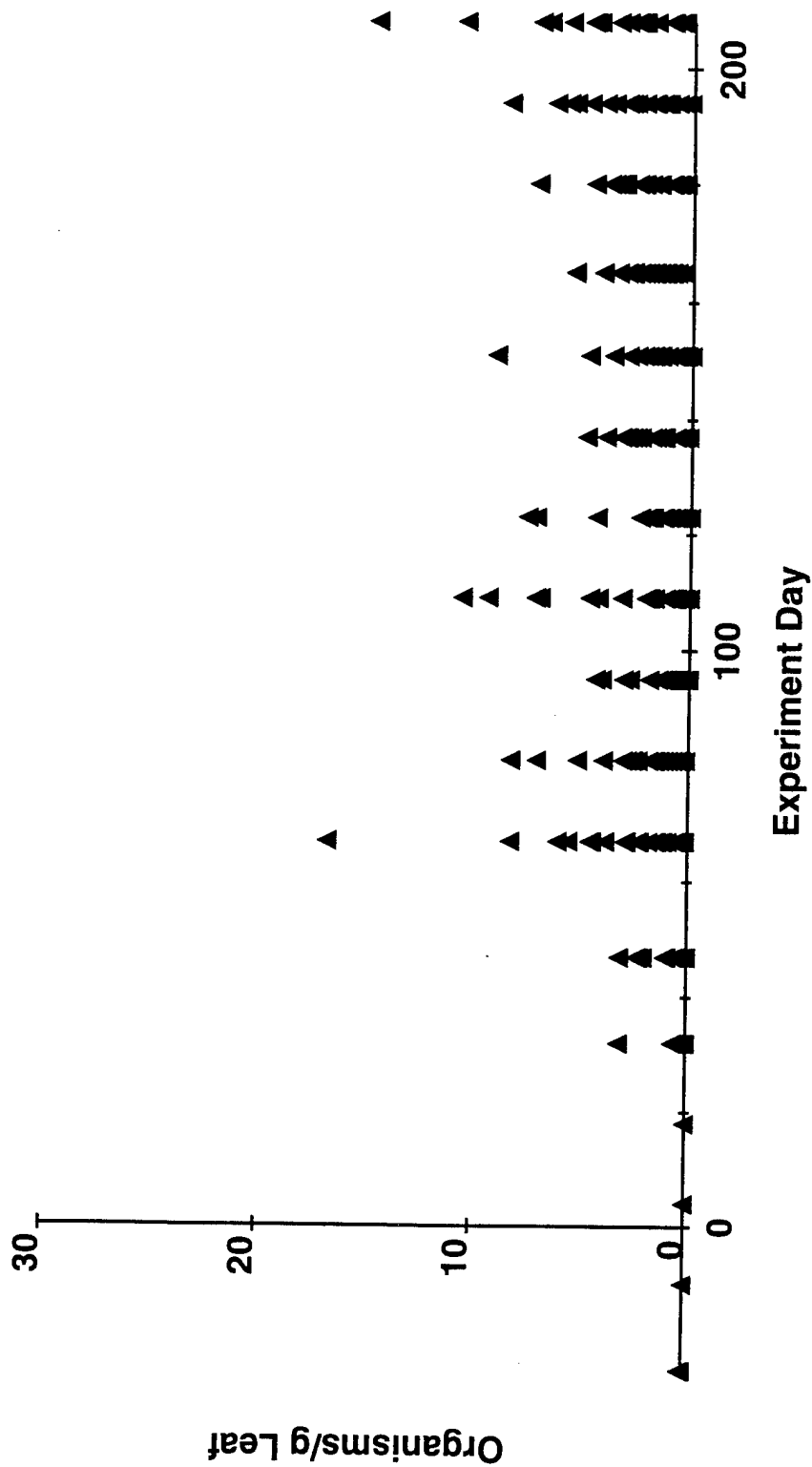
Figure 45c. Molluscs
Snails - Low Dose
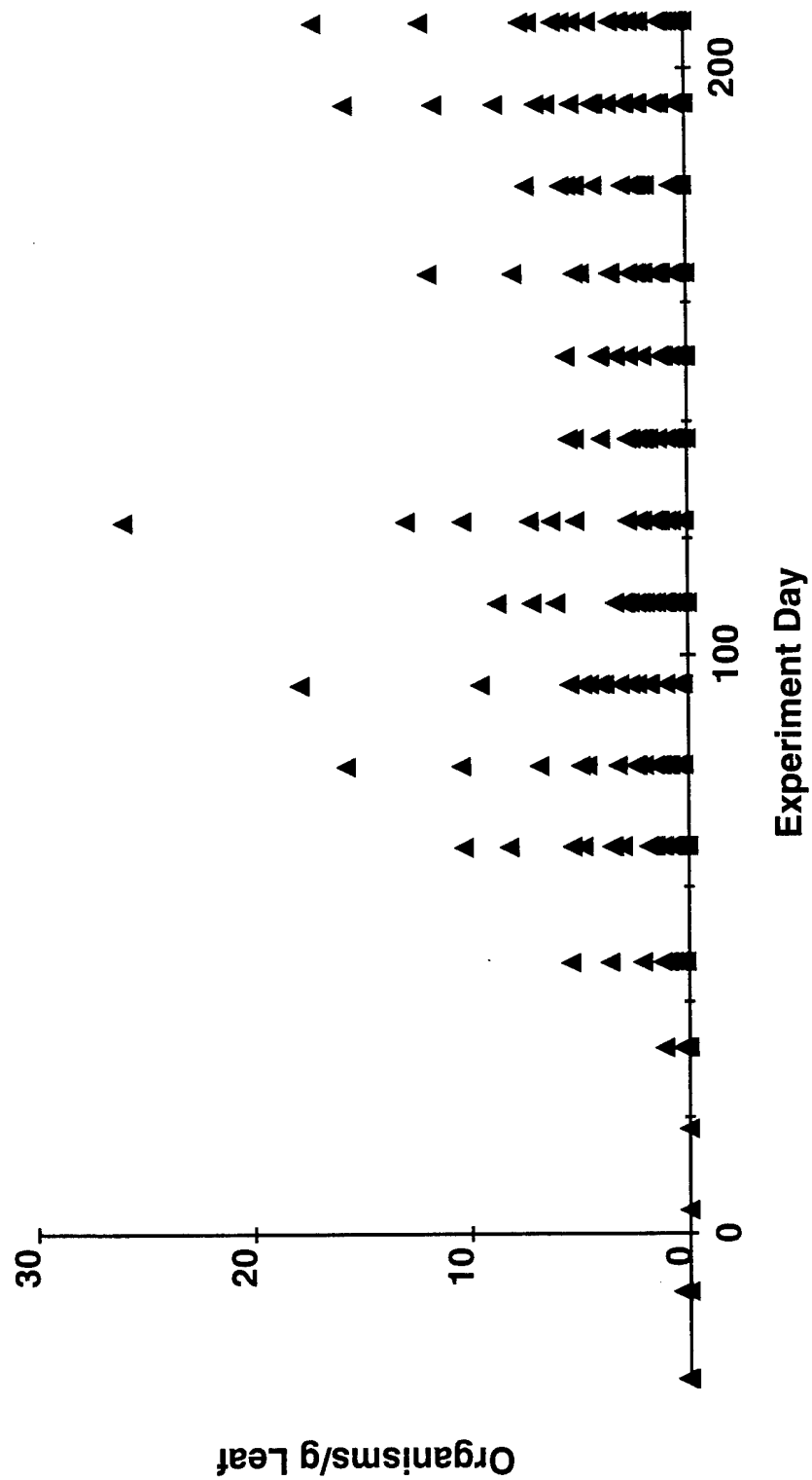
Figure 45d. Molluscs
Snails - Undosed

Organisms/g Leaf

Experiment Day
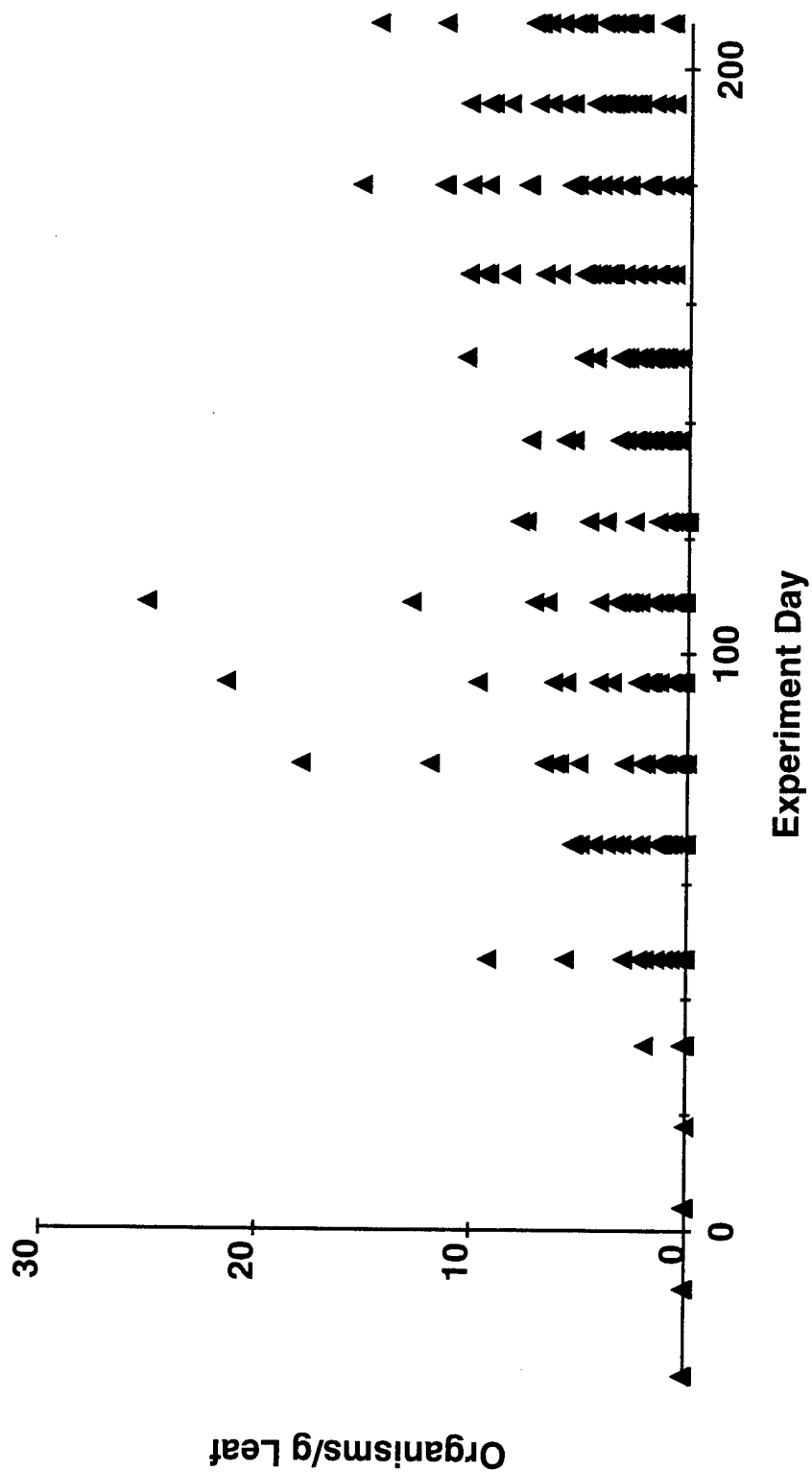
Figure 46. Periphyton and Leaf Pack Correlations
Amphipods vs. Snails
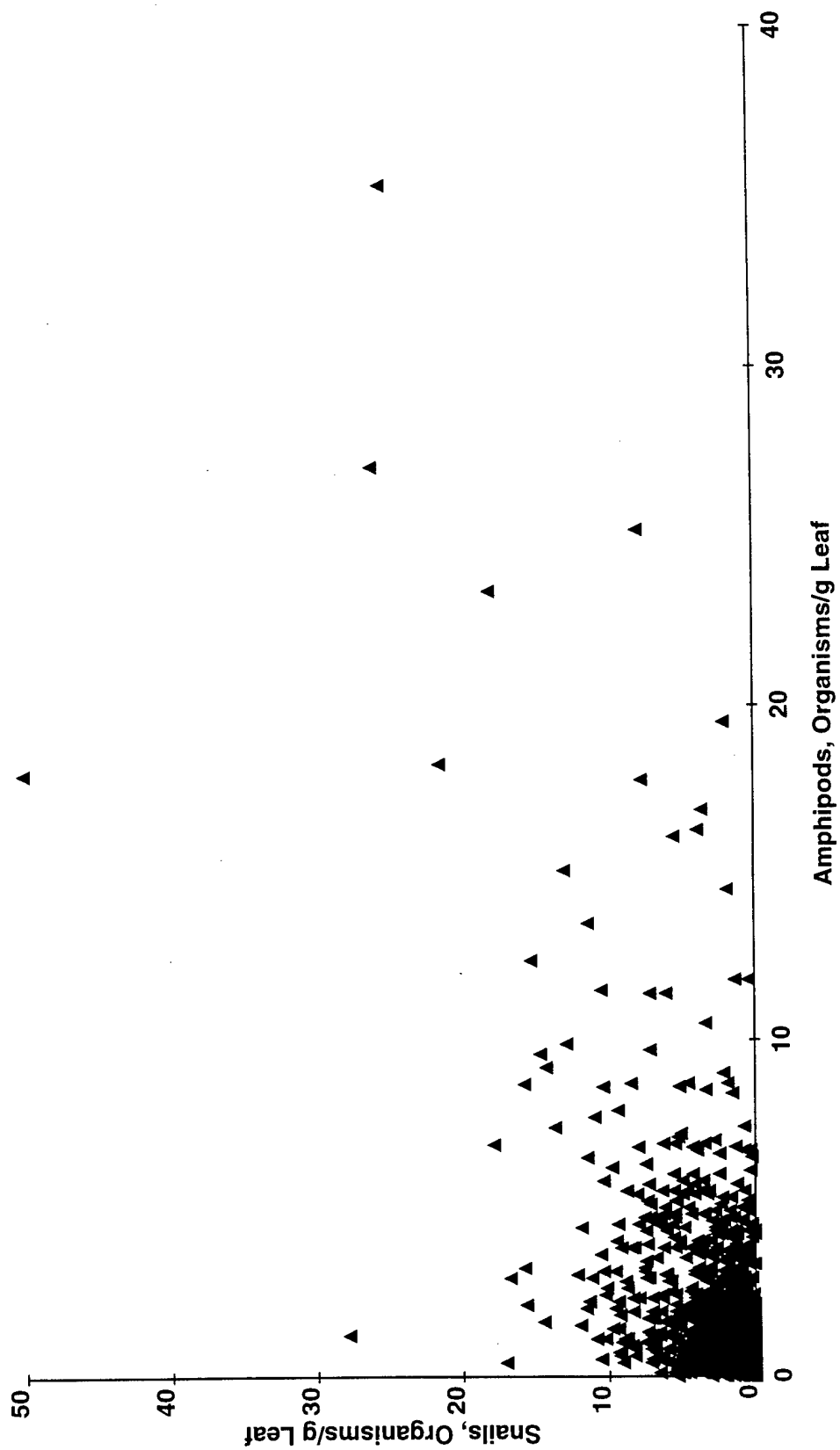
Figure 47. Periphyton and Leaf Pack Correlations
Ankistrodesmus vs. Pennate Diatoms

Ankistrodesmus, Cells/mL

Pennate Diatoms, Cells/mL
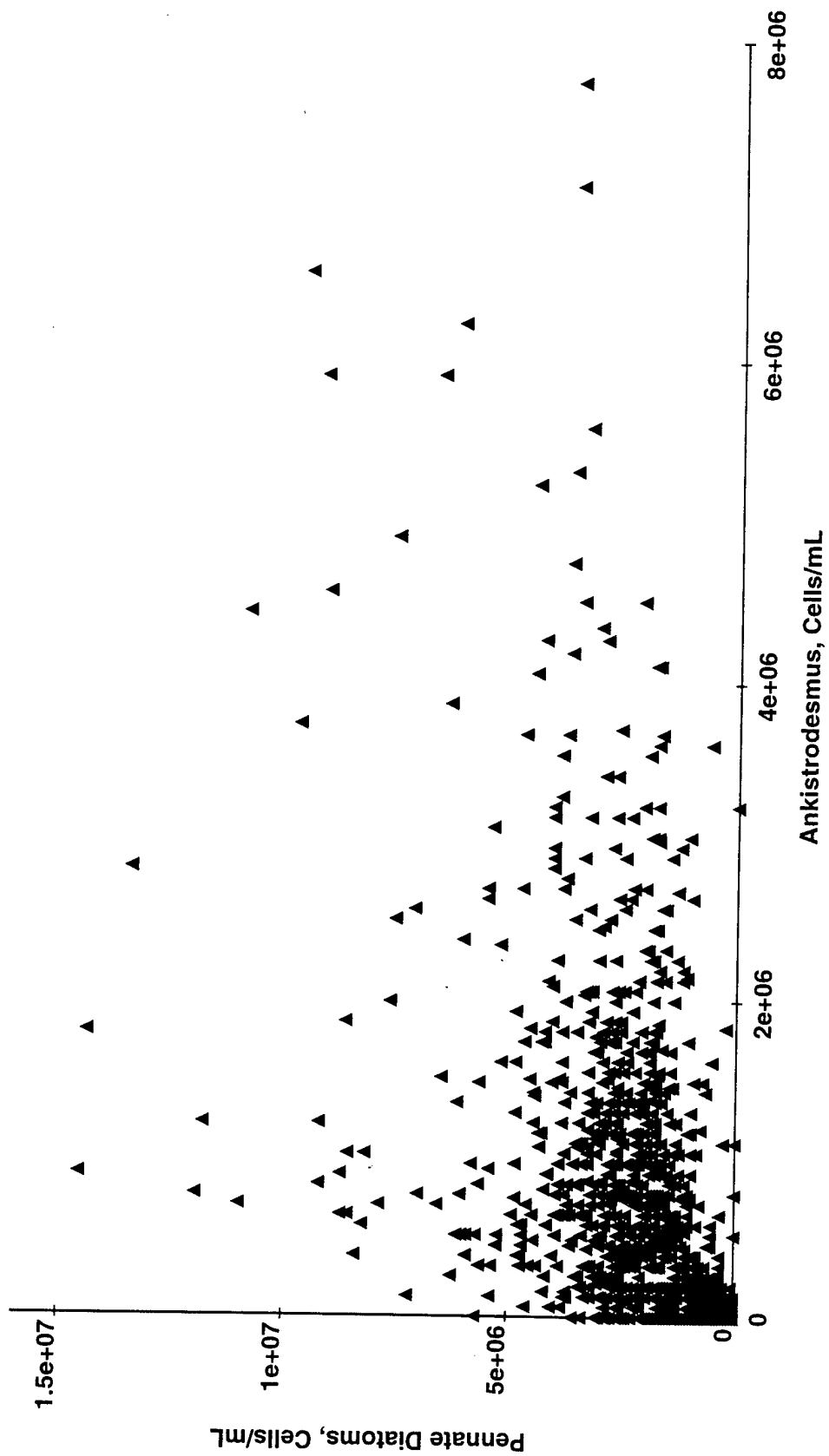
Figure 48. Periphyton and Leaf Pack Correlations
Ankistrodesmus vs. Schroederia
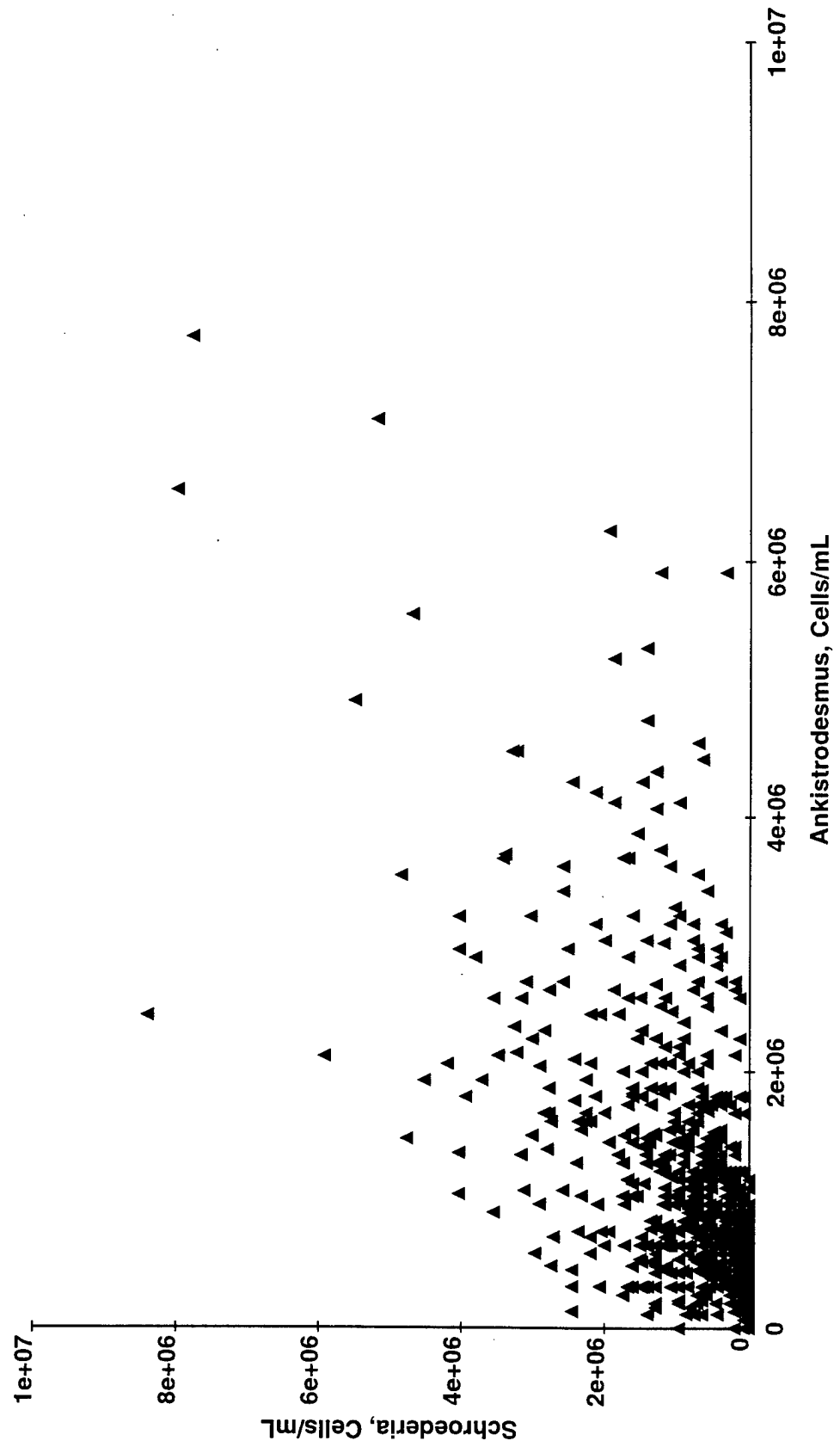
Figure 49. Periphyton and Leaf Pack Correlations
Ankistrodesmus vs. Snails
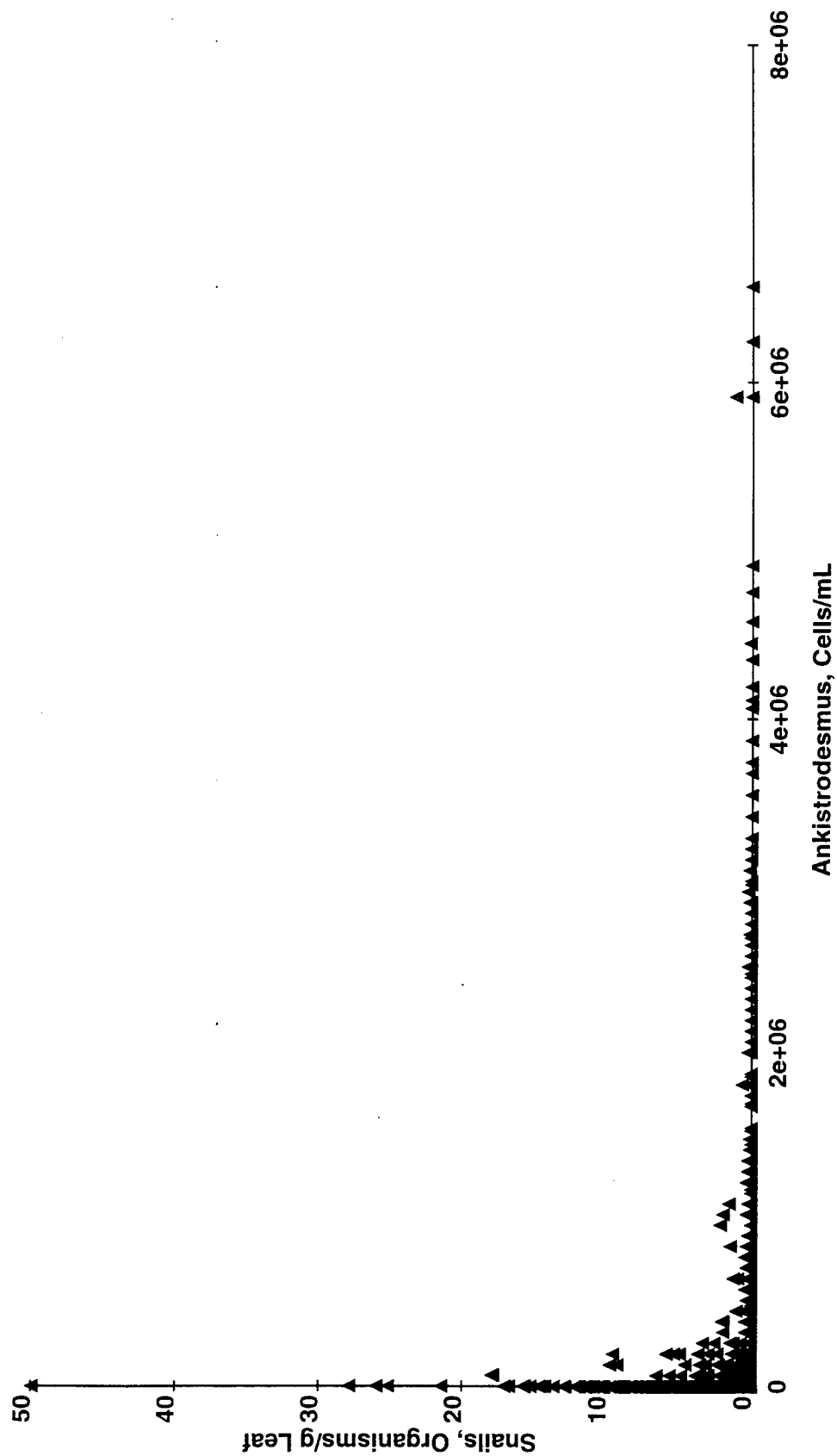
Figure 50. Periphyton and Leaf Pack Correlations
Autotrophic Flagellates vs. Heterotrophic Flagellates
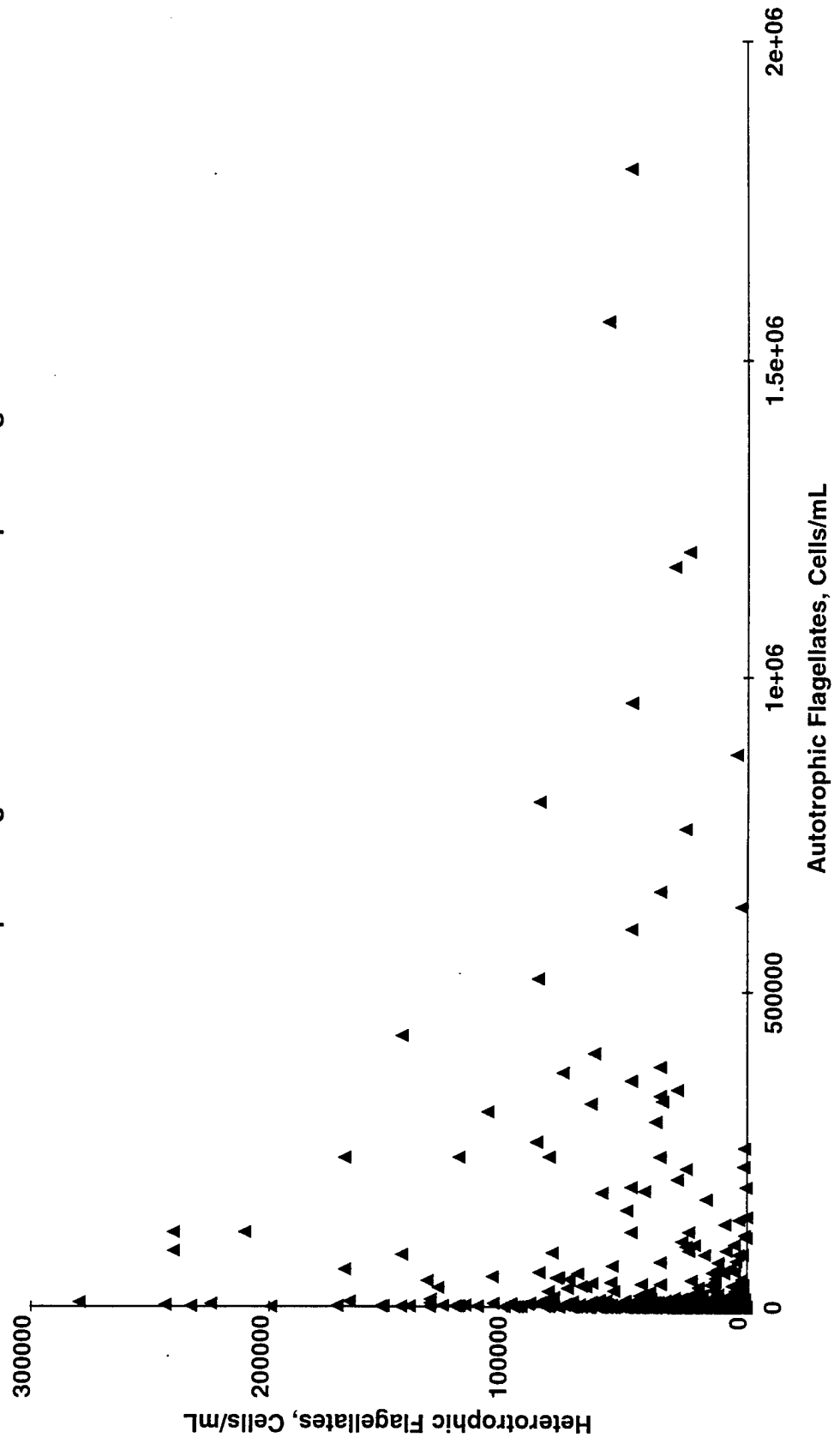
Figure 51. Periphyton and Leaf Pack Correlations
Filamentous Greens vs. Autotrophic Flagellates
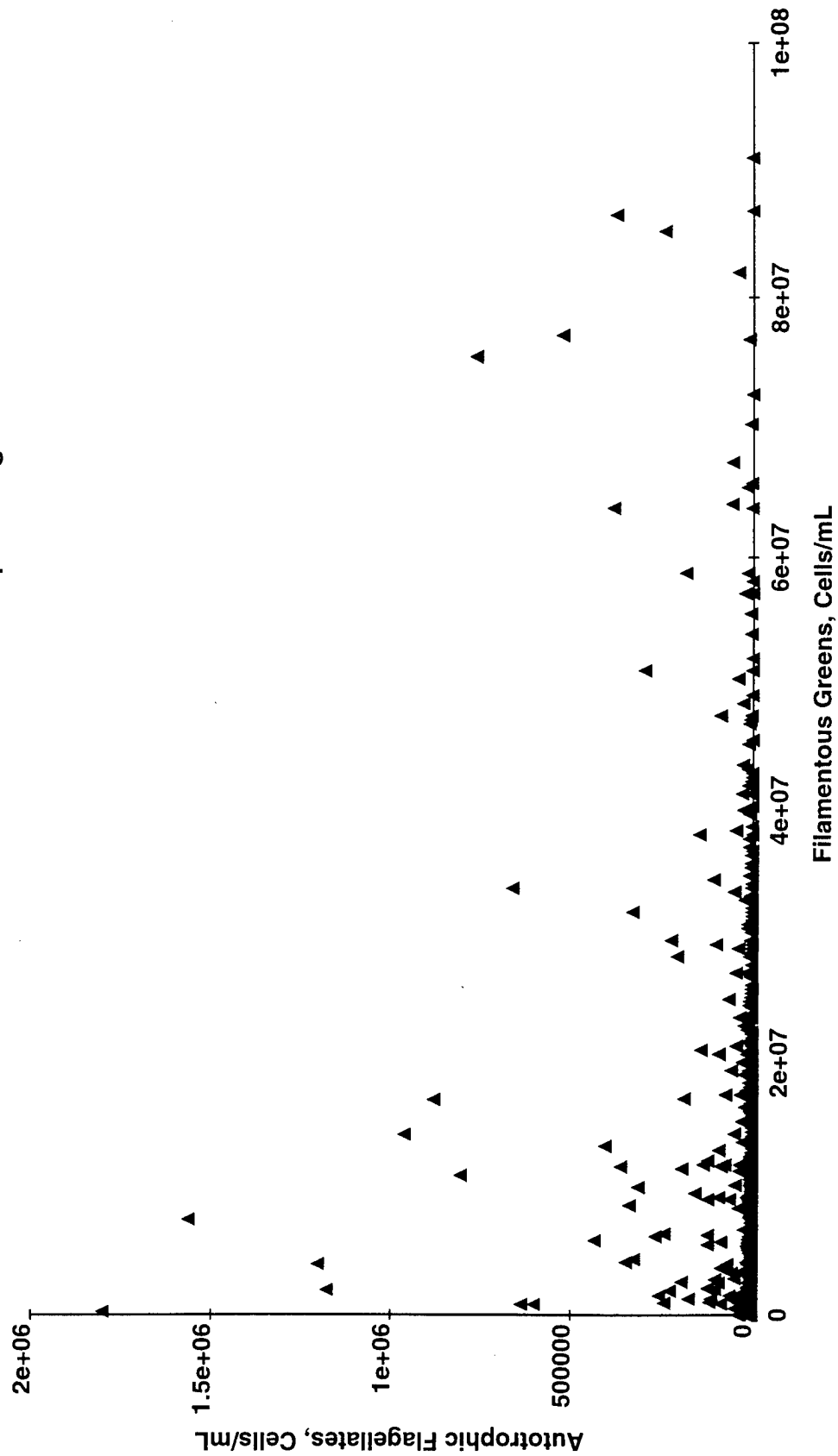
Figure 52. Periphyton and Leaf Pack Correlations
Filamentous Greens vs. Nostoc
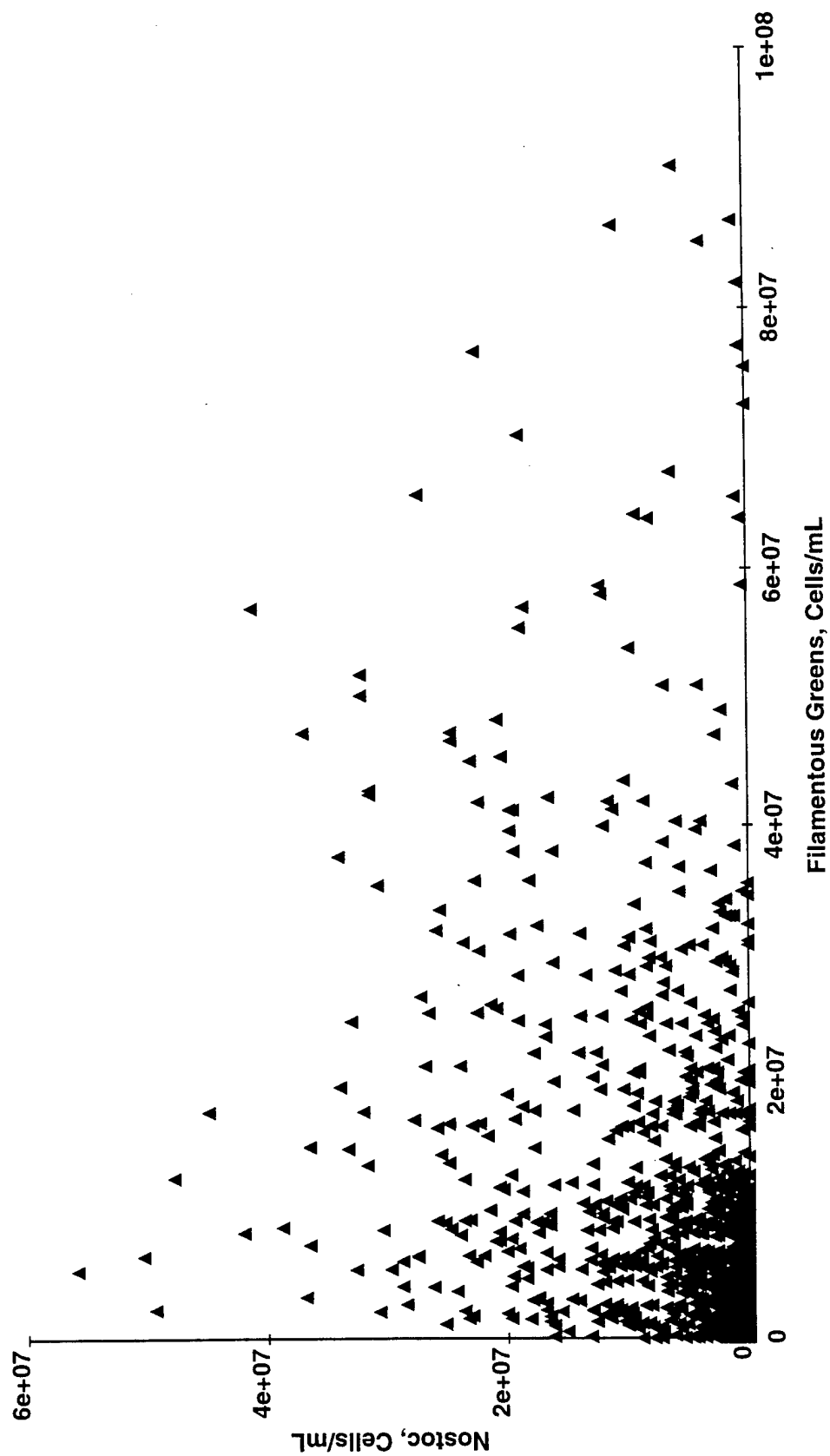
Figure 53. Periphyton and Leaf Pack Correlations
Pennate Diatoms vs. TTC Respiration
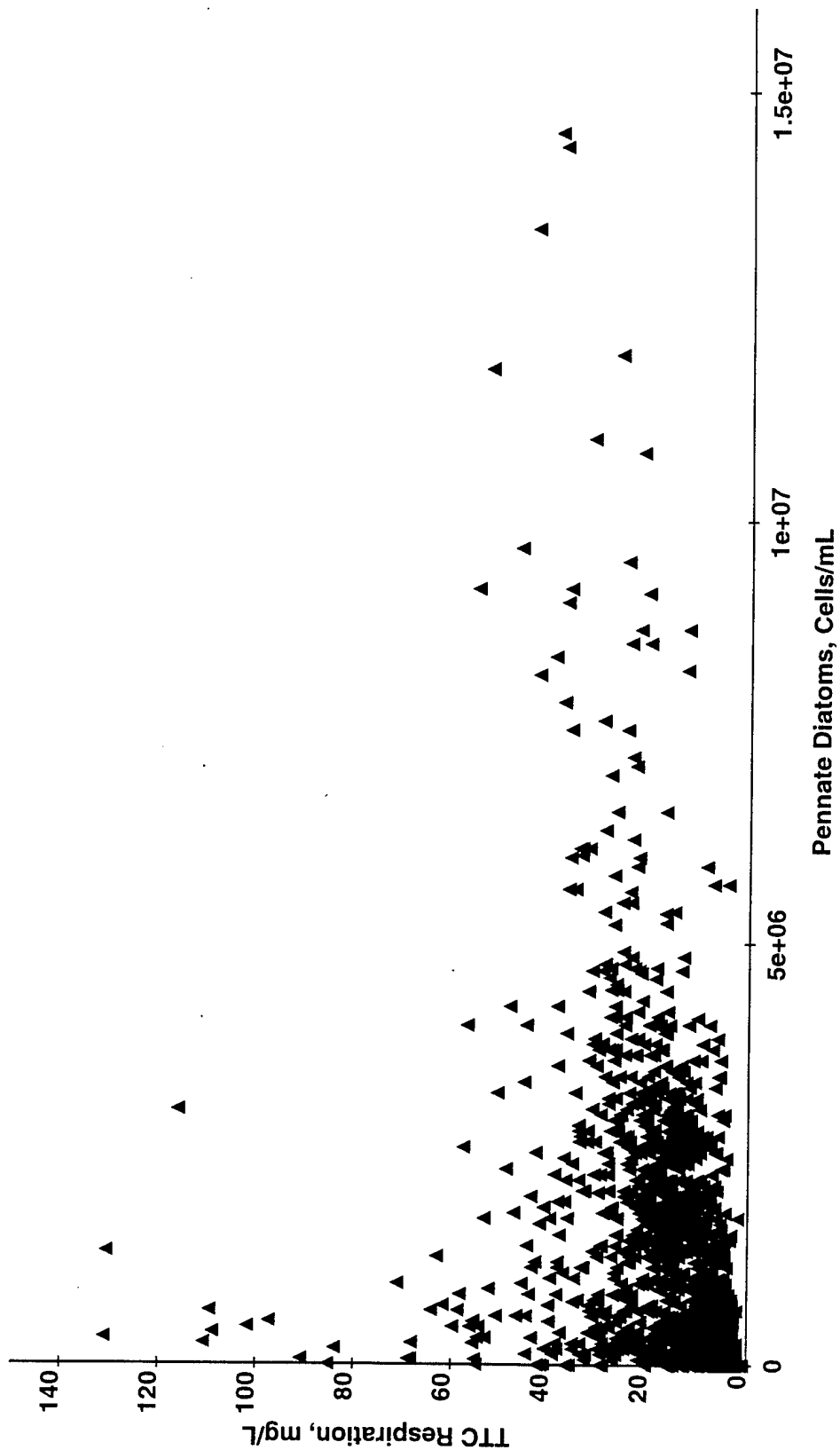
Figure 54. Periphyton and Leaf Pack Correlations
Schroederia vs. Pennate Diatoms
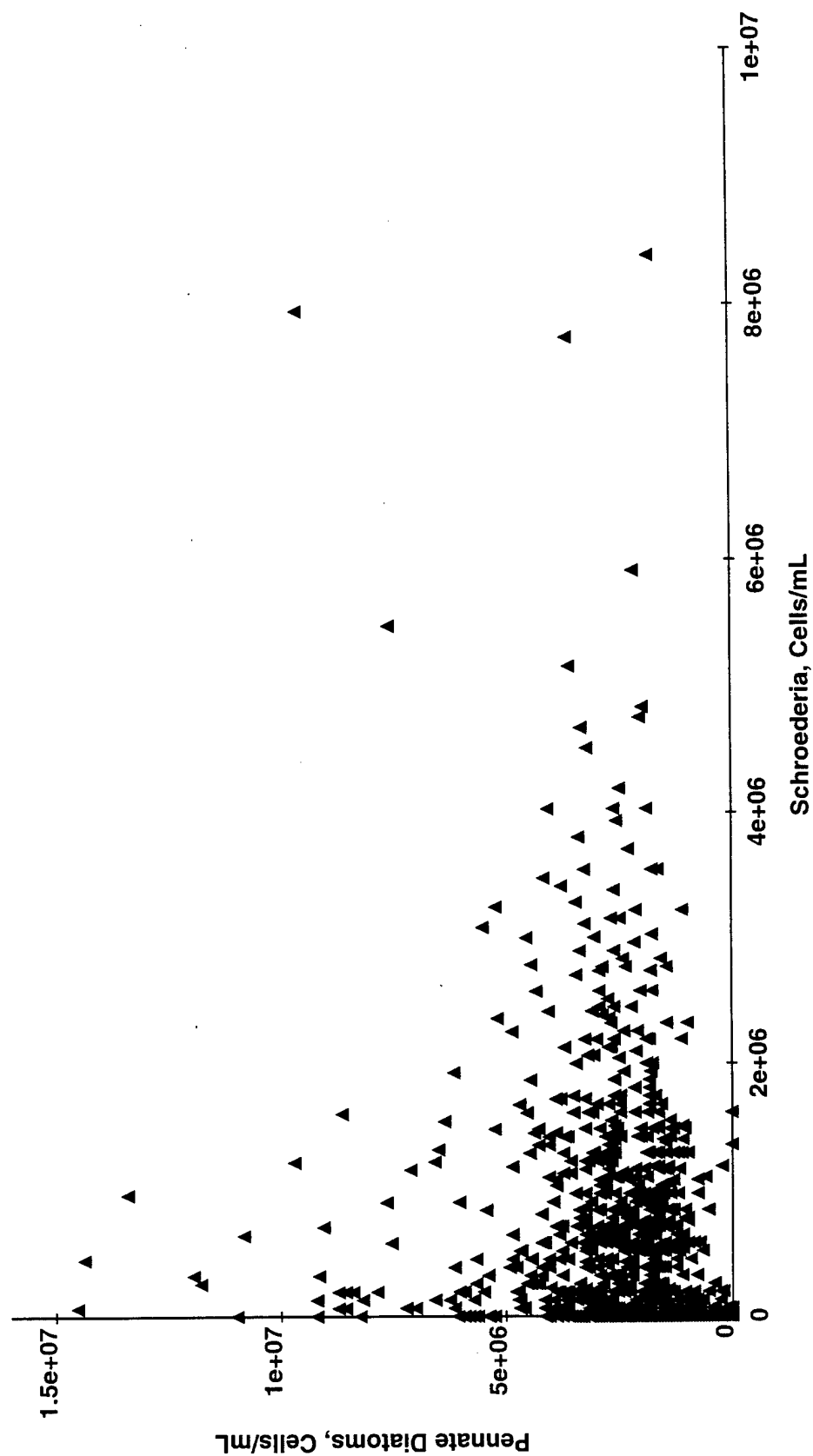
Figure 55. Periphyton and Leaf Pack Correlations
Schroederia vs. Snails

Snails, Organisms/g Leaf

Schroederia, Cells/mL
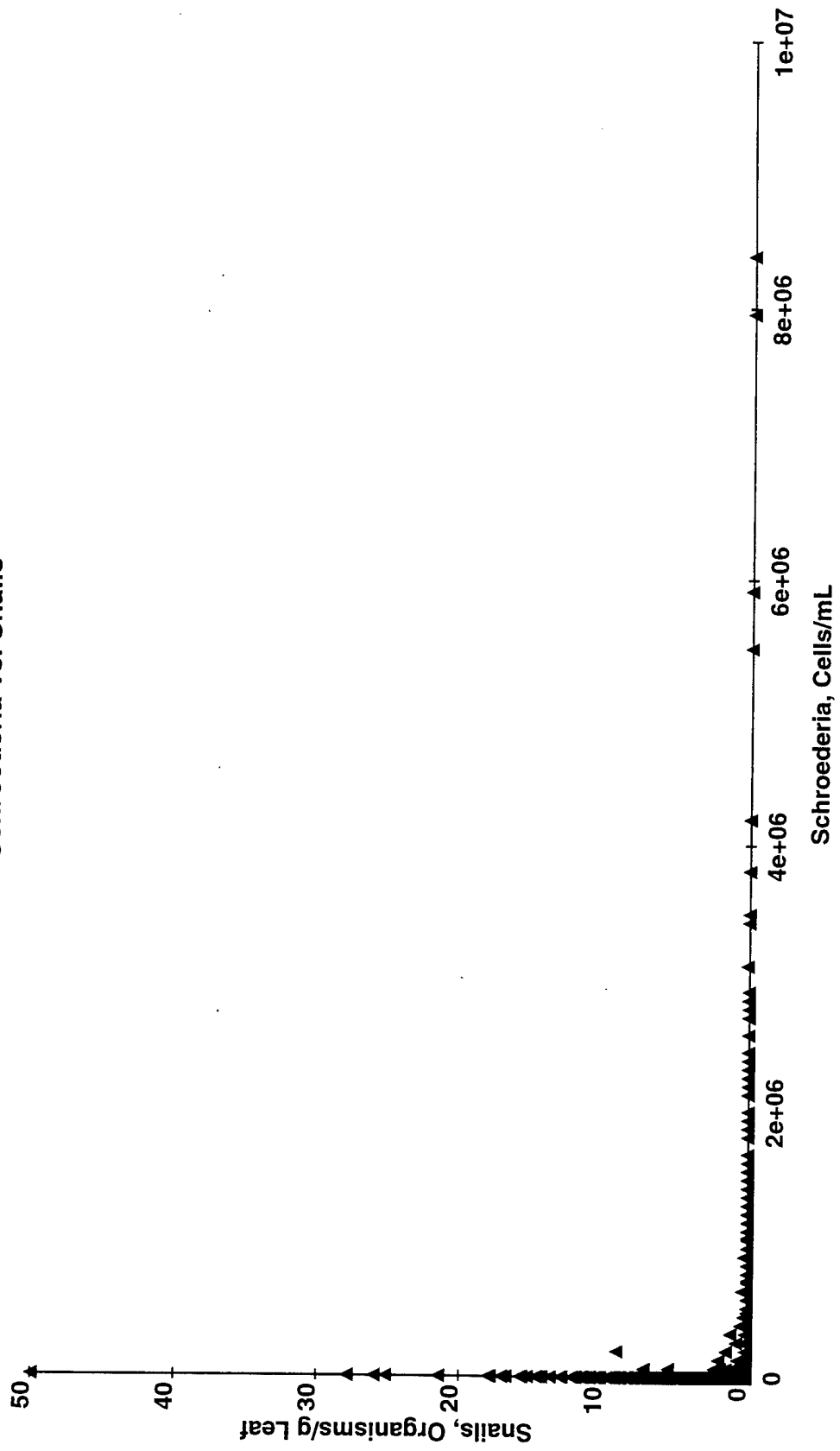
Figure 56. Periphyton and Leaf Pack Correlations
Total Solids vs. Ash-Free Dry Weight
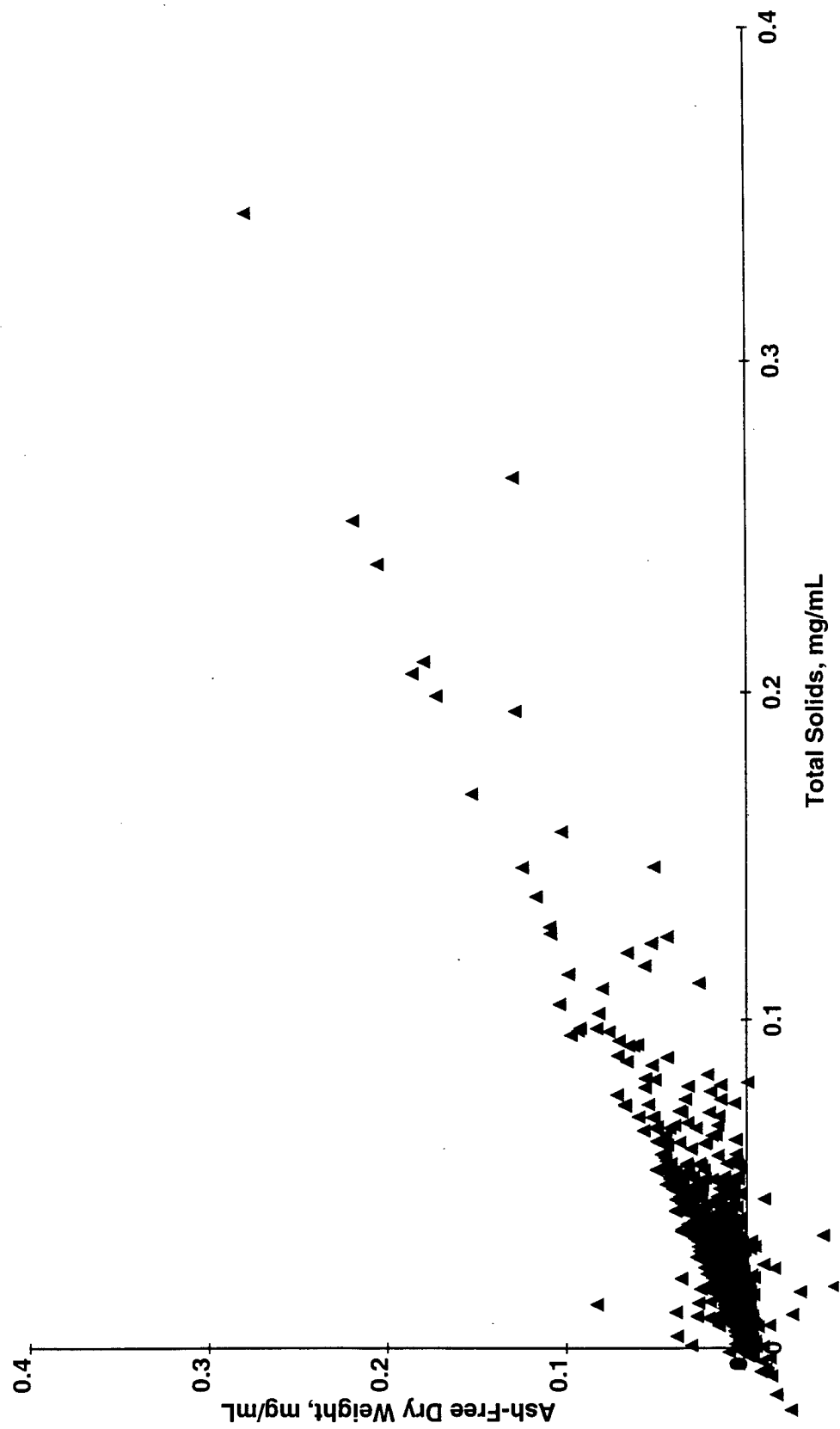
Figure 57. Periphyton and Leaf Pack Correlations
Total Solids vs. Total Volatile Solids
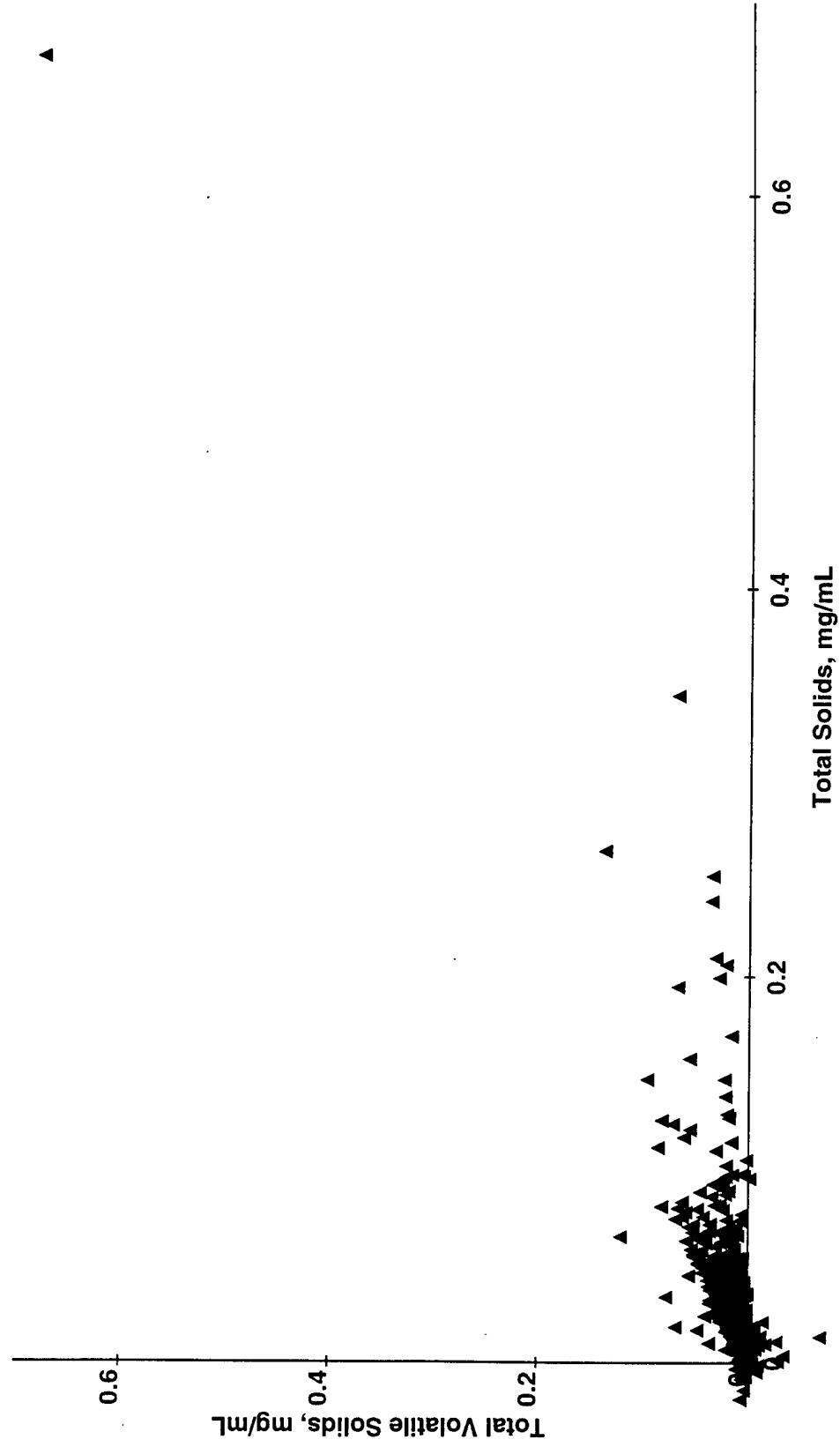
Figure 58. Periphyton and Leaf Pack Correlations
Total Volatile Solids vs. TTC Respirations
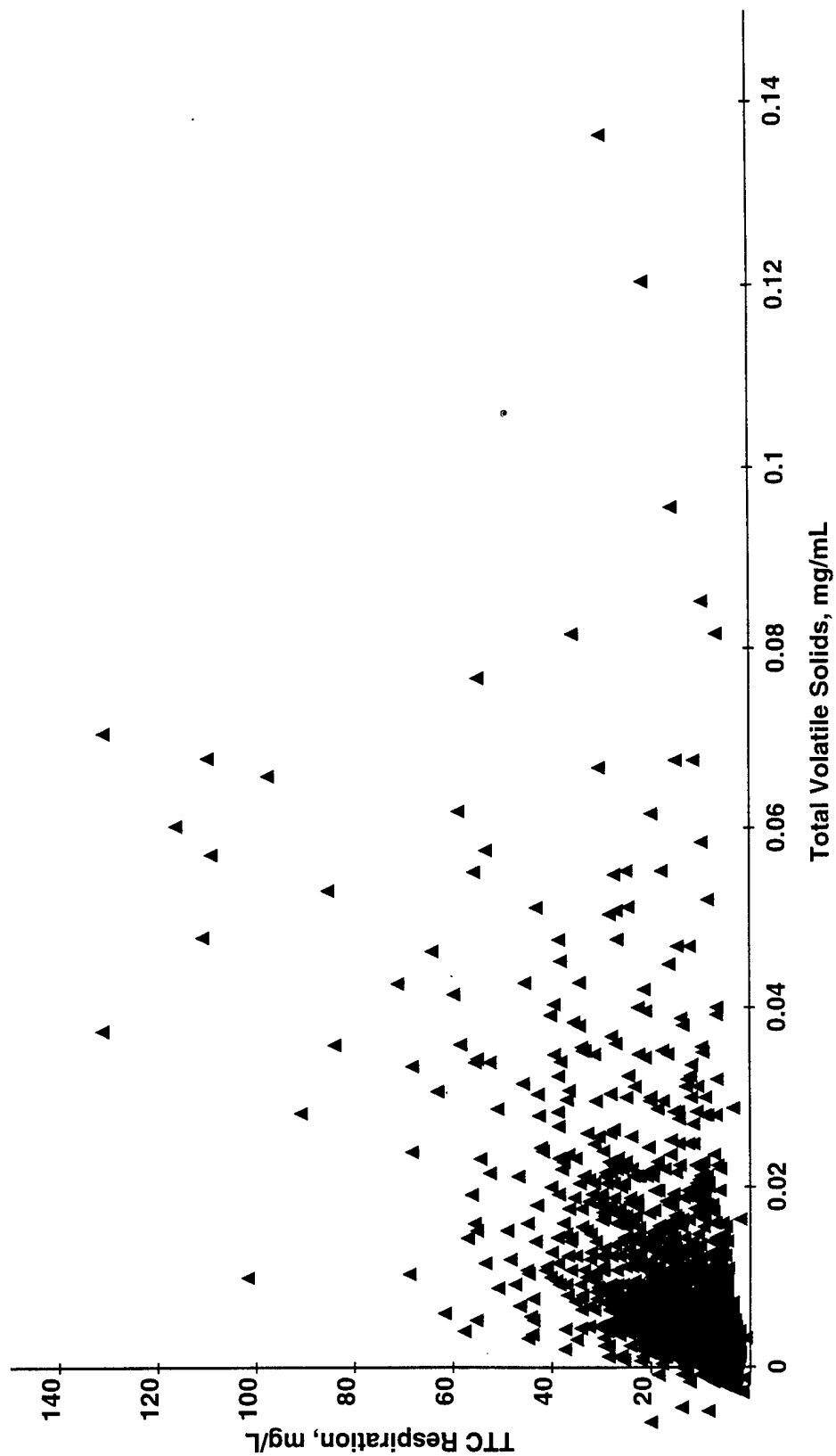
Figure 59. Periphyton and Leaf Pack Correlations
Total Volatile Solids vs. Chlorophyll
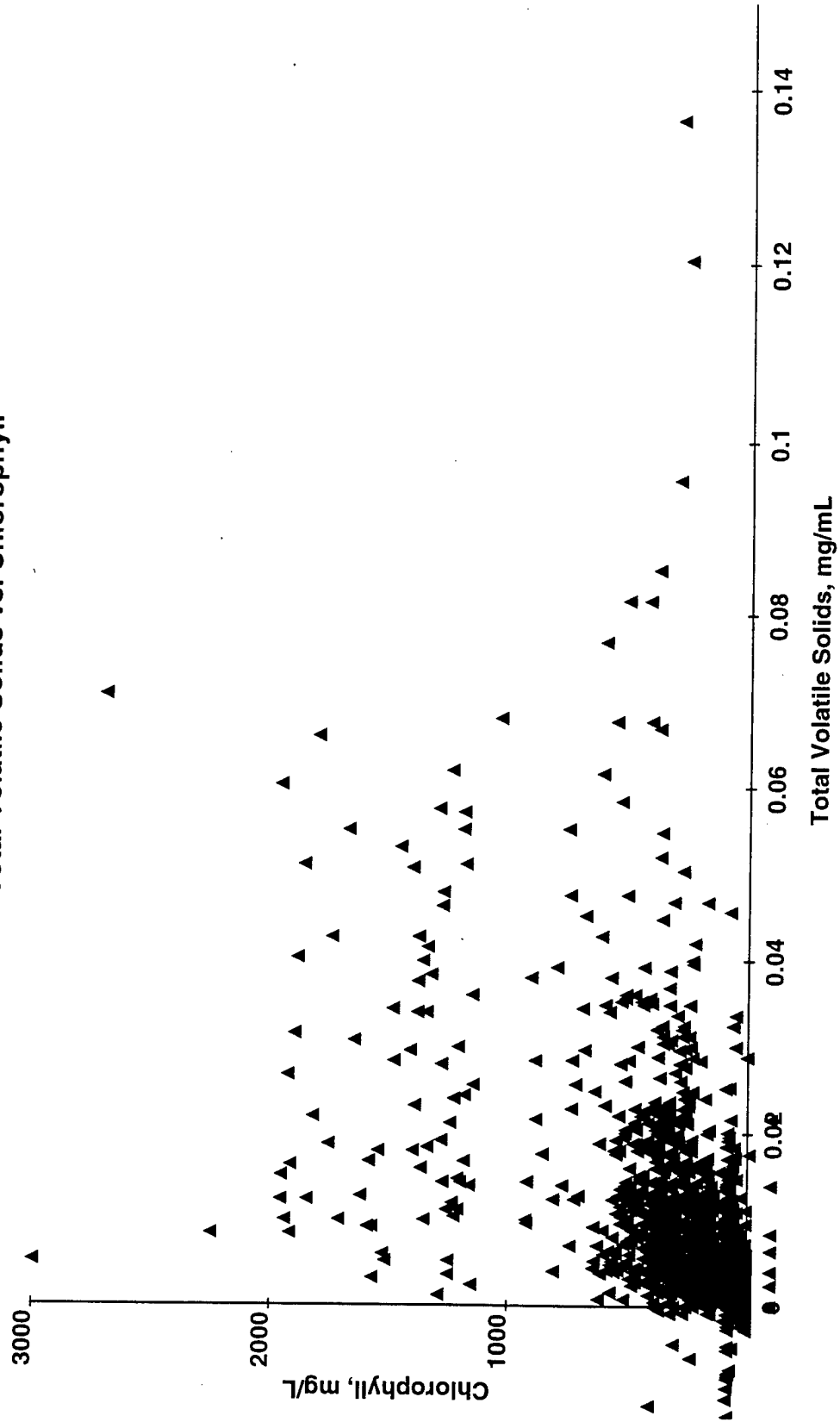
Figure 60. Periphyton and Leaf Pack Correlations
TTC Respiration vs. Chlorophyll
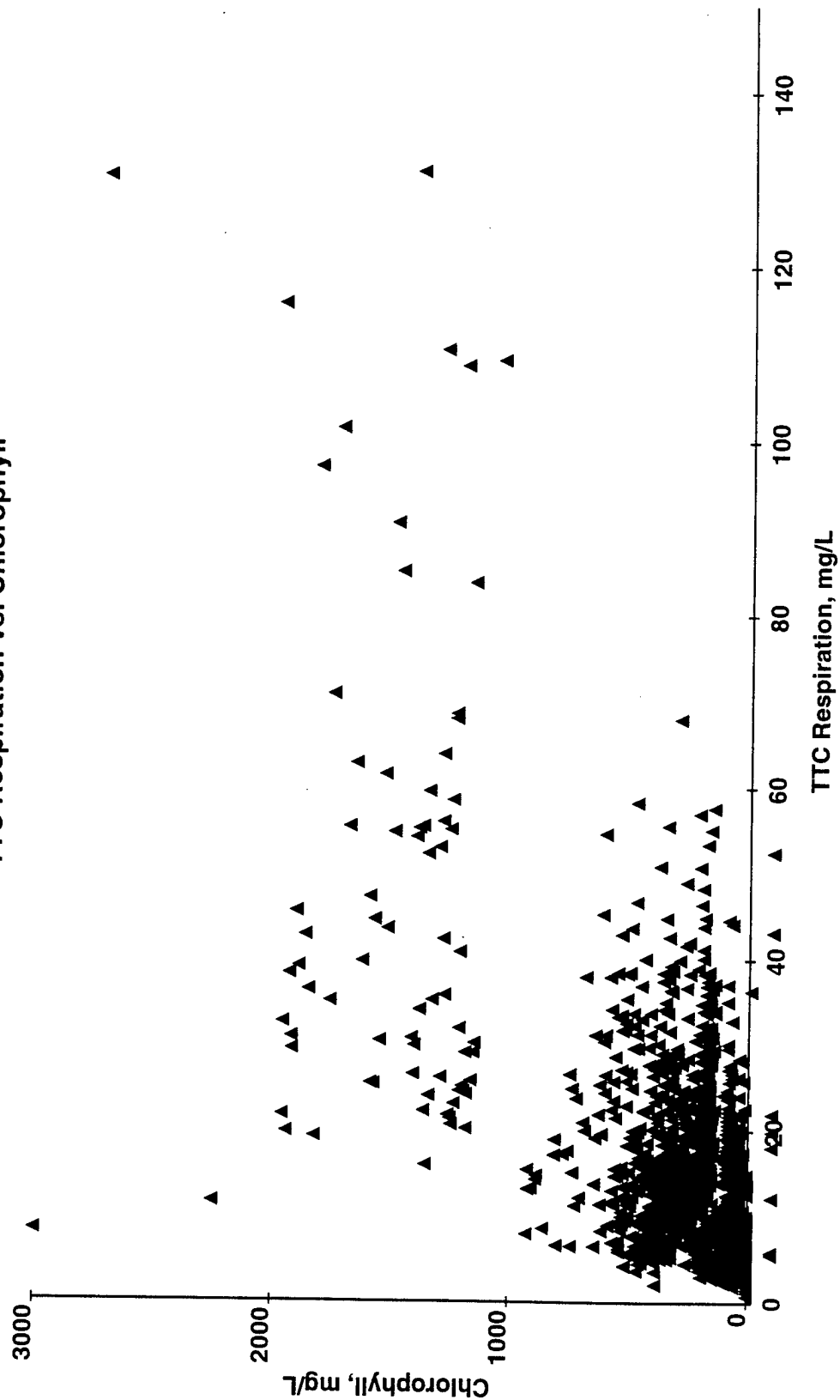
Figure 61a. Phytoplankton - Ciliates
Other - High Dose
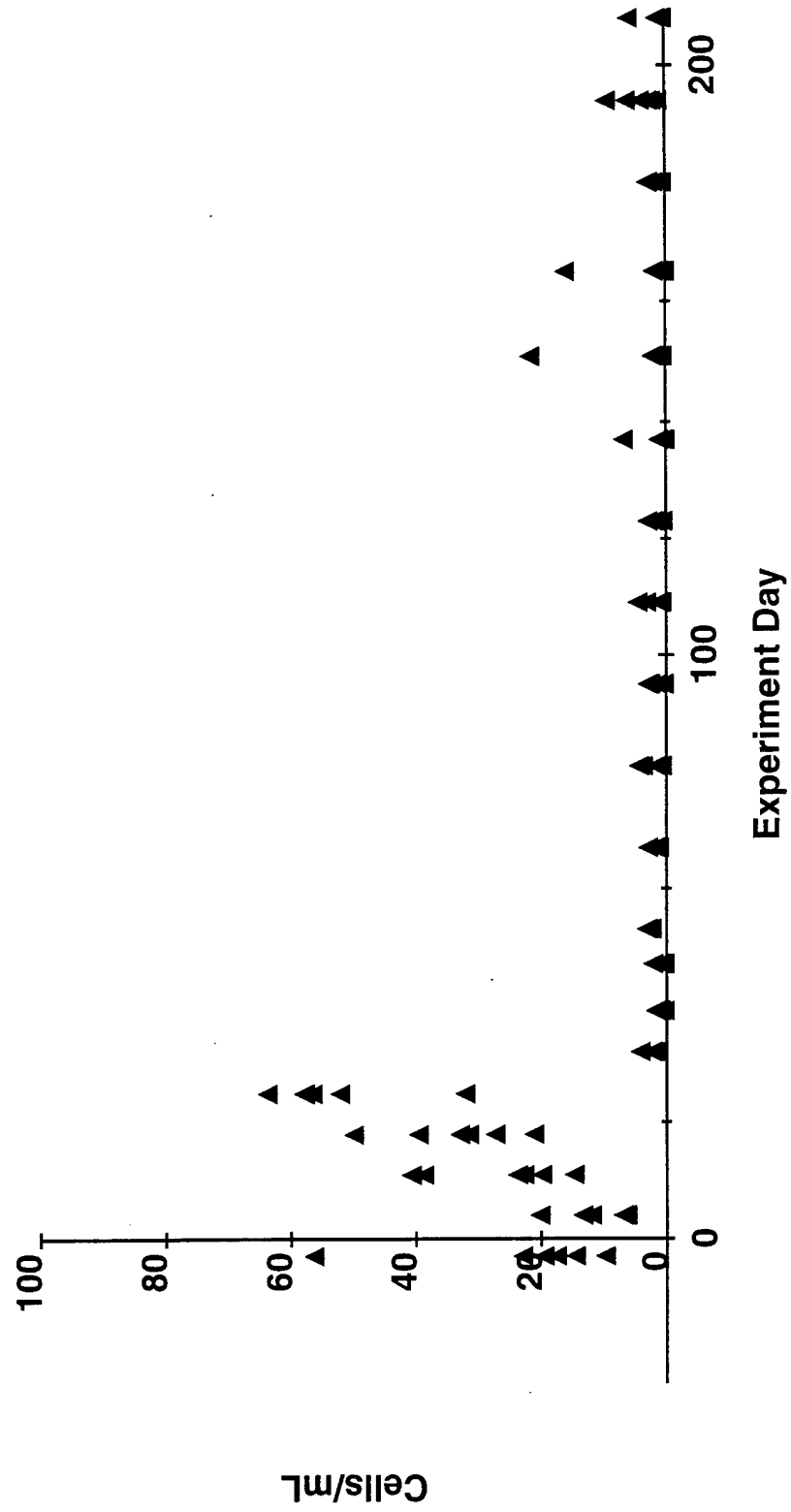
Figure 61b. Phytoplankton - Ciliates
Other - Mid-dose
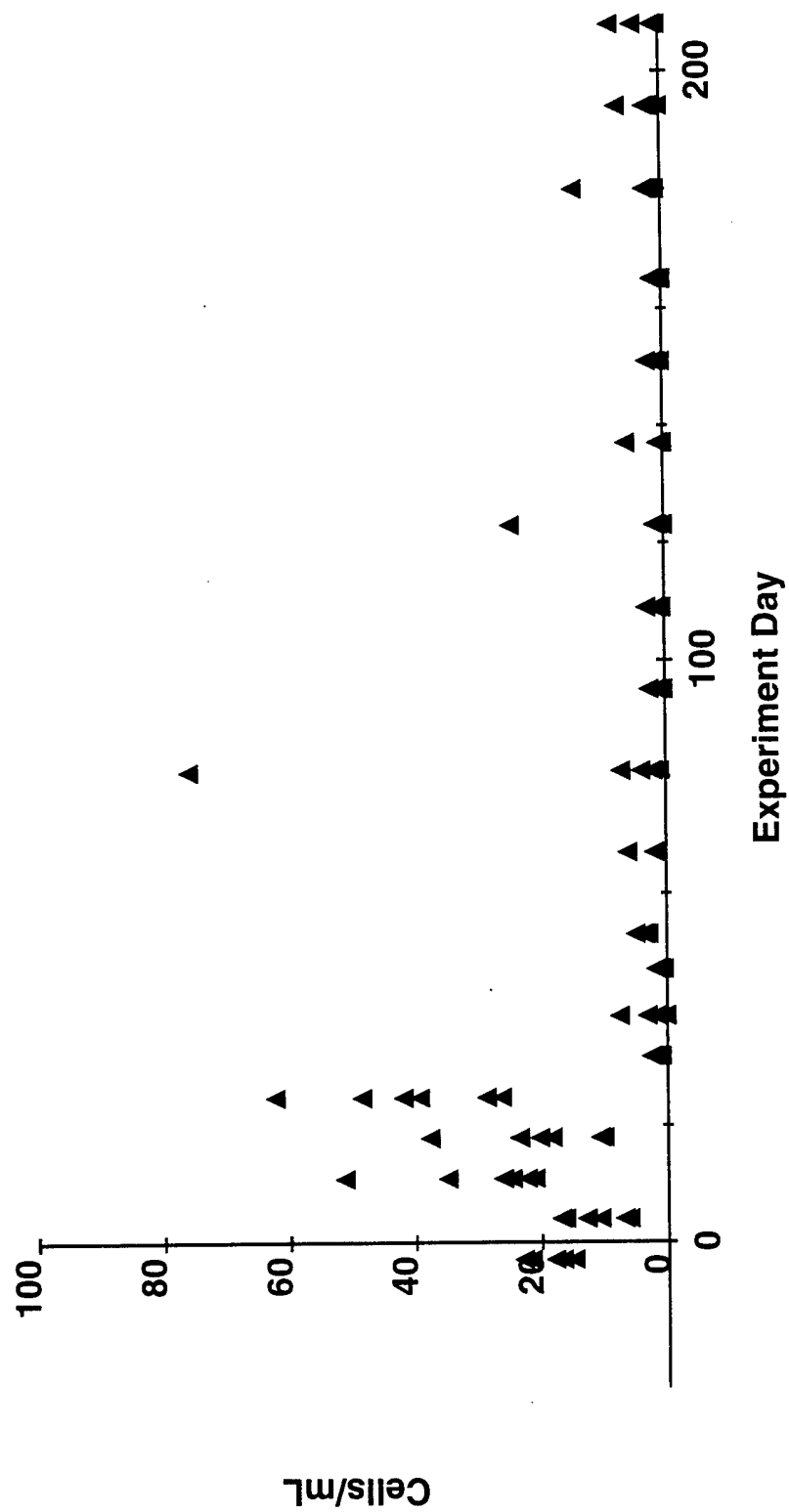
Figure 61c. Phytoplankton - Ciliates
Other - Low Dose
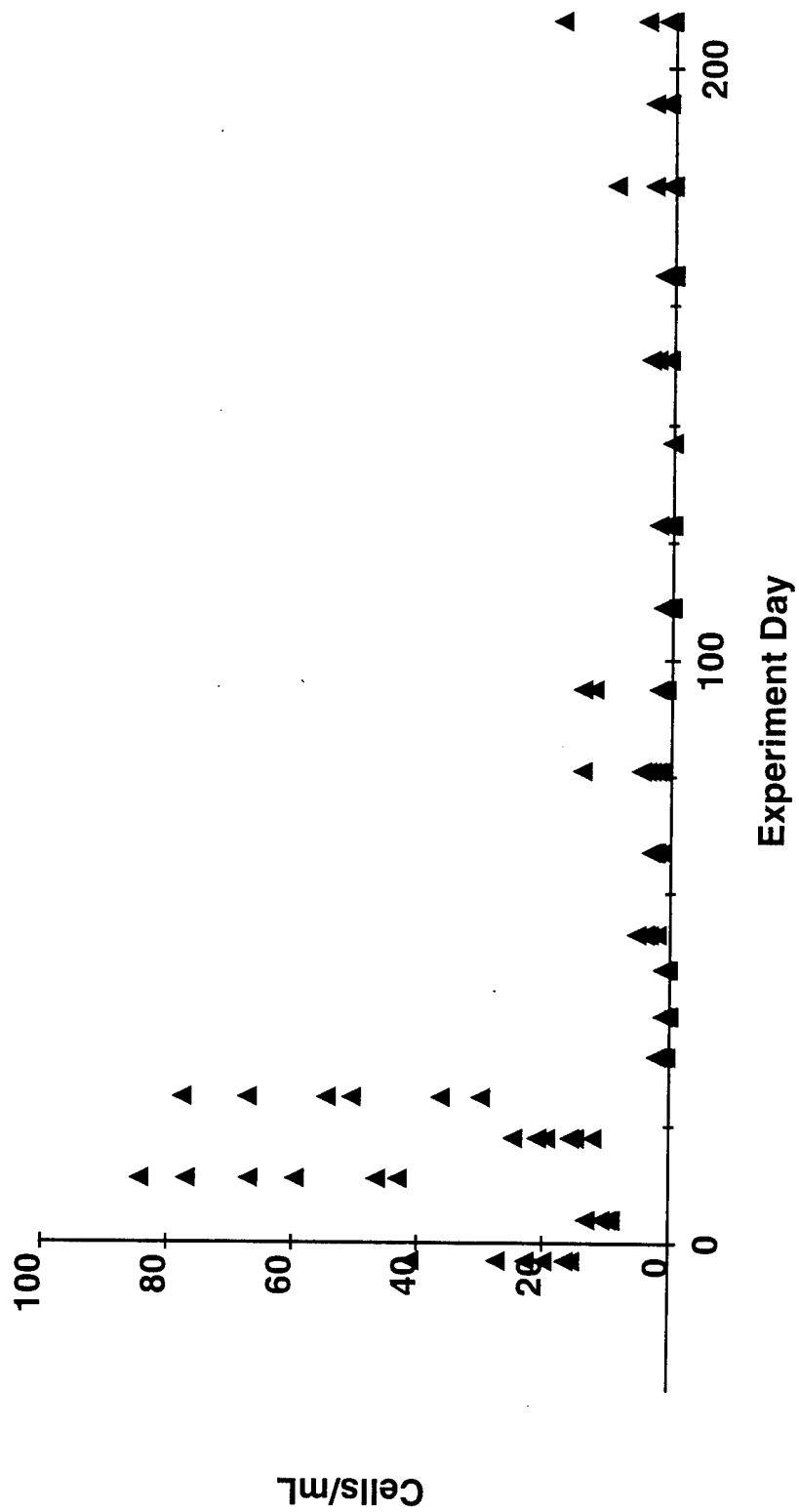
Figure 61d. Phytoplankton - Ciliates
Other - Undosed
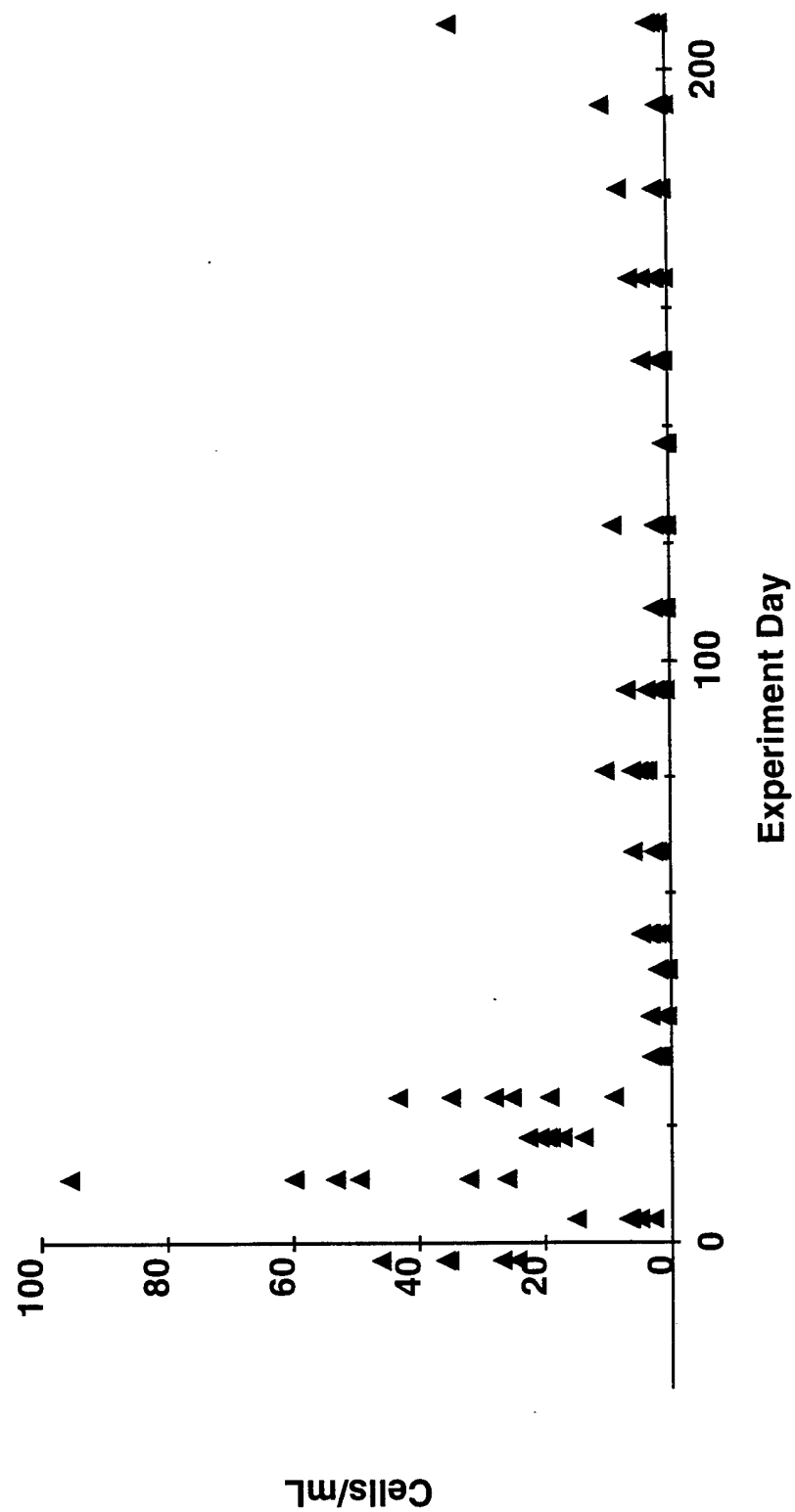
Figure 62a. Phytoplankton - Cyanophytes
Nostoc - High Dose
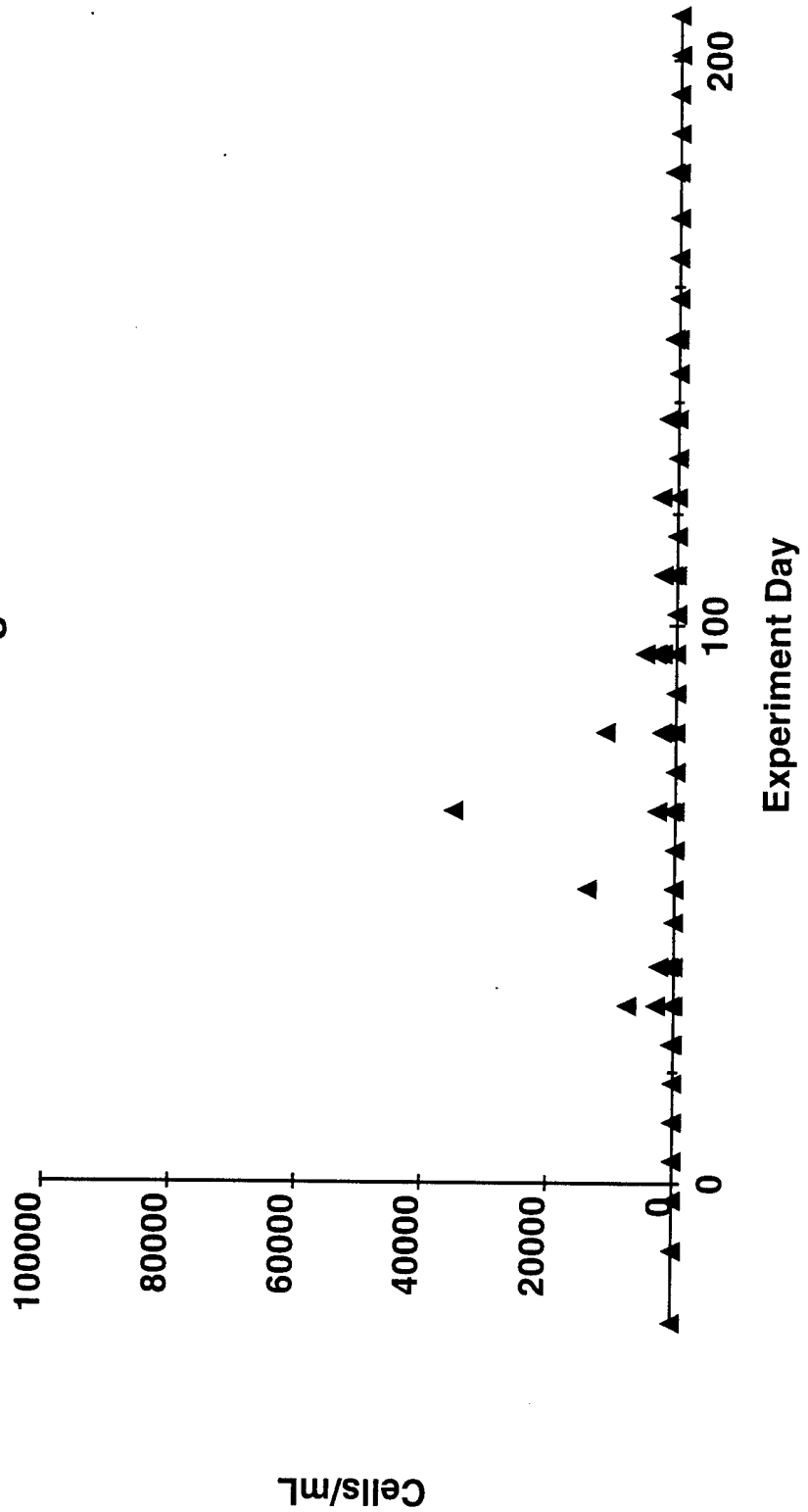
Figure 62b. Phytoplankton - Cyanophytes
Nostoc - Mid-dose

Cells/mL

Experiment Day
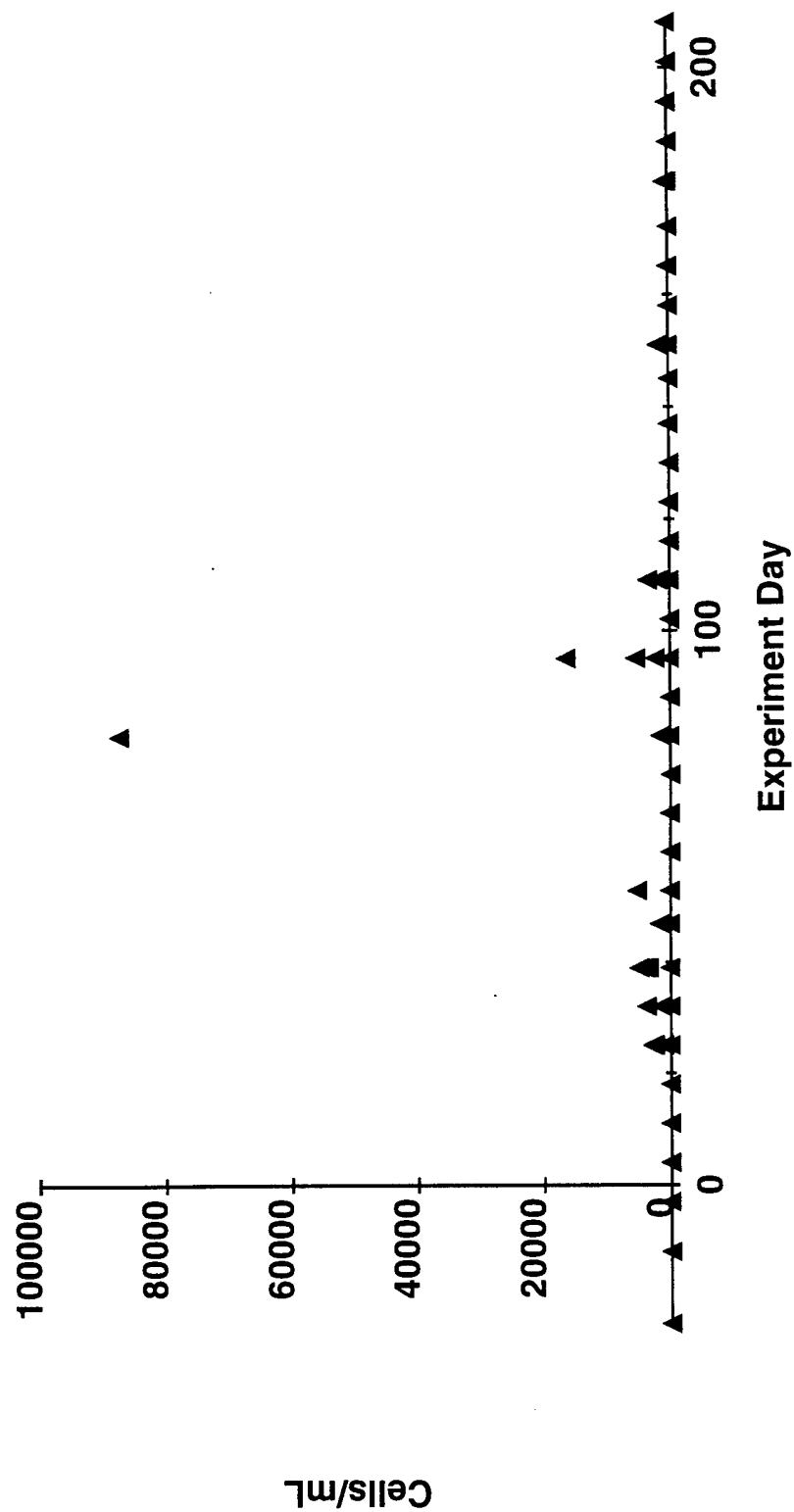
Figure 62c. Phytoplankton - Cyanophytes
Nostoc - Low Dose
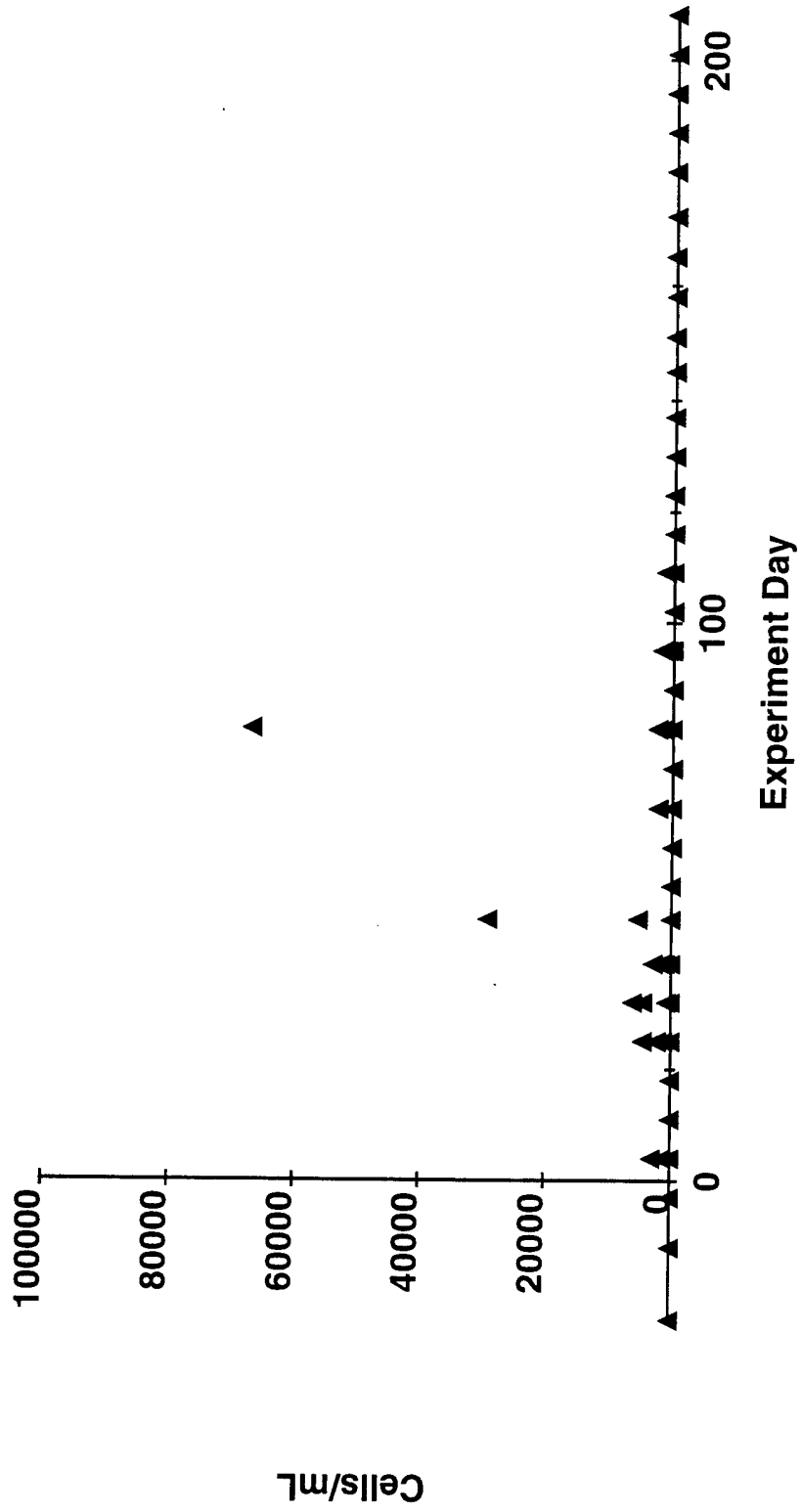
Figure 62d. Phytoplankton - Cyanophytes
Nostoc - Undosed
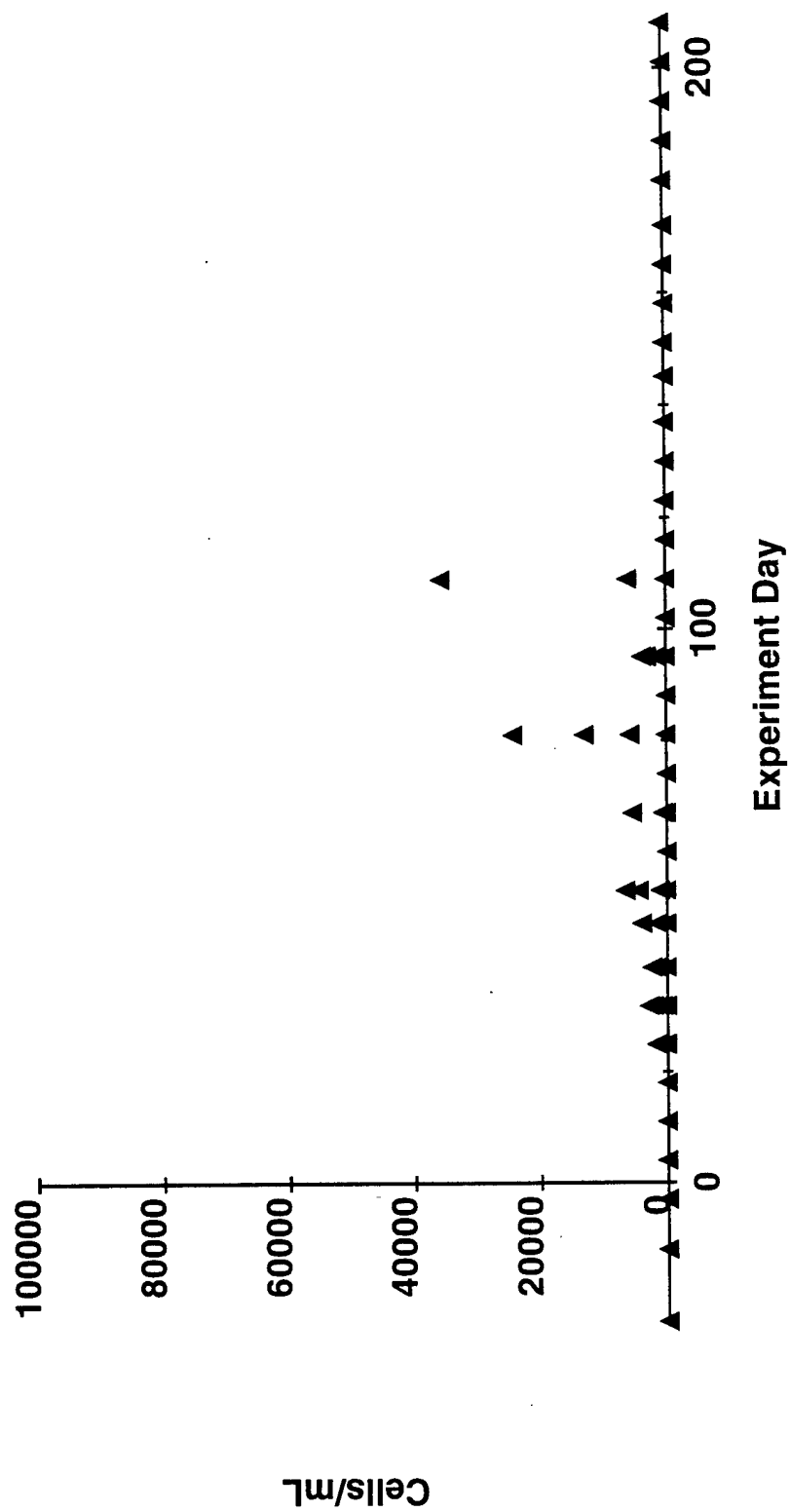
Figure 63a. Phytoplankton - Cyanophytes
Oscillatoria - High Dose
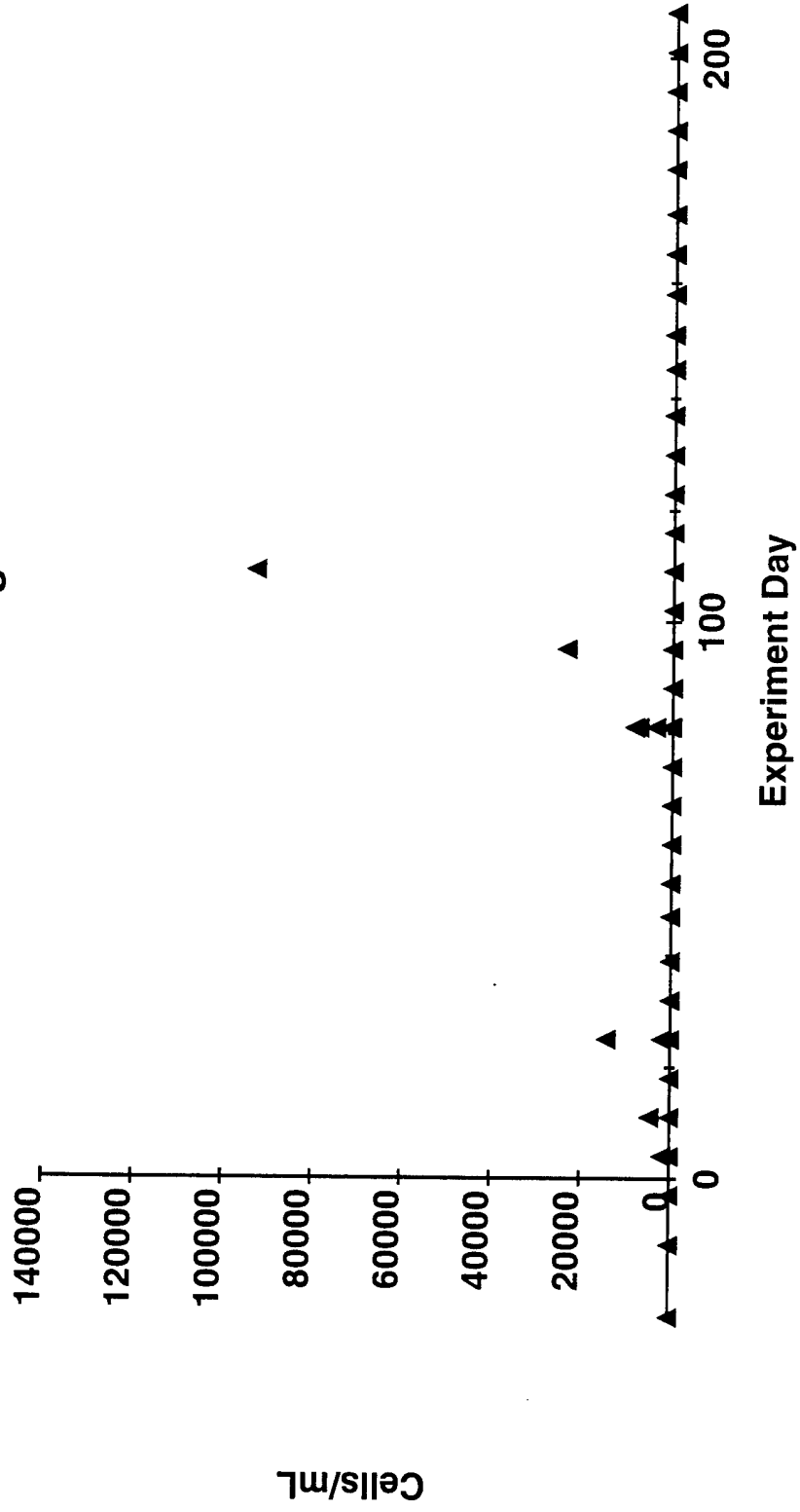
Figure 63b. Phytoplankton - Cyanophytes
Oscillatoria - Mid-dose
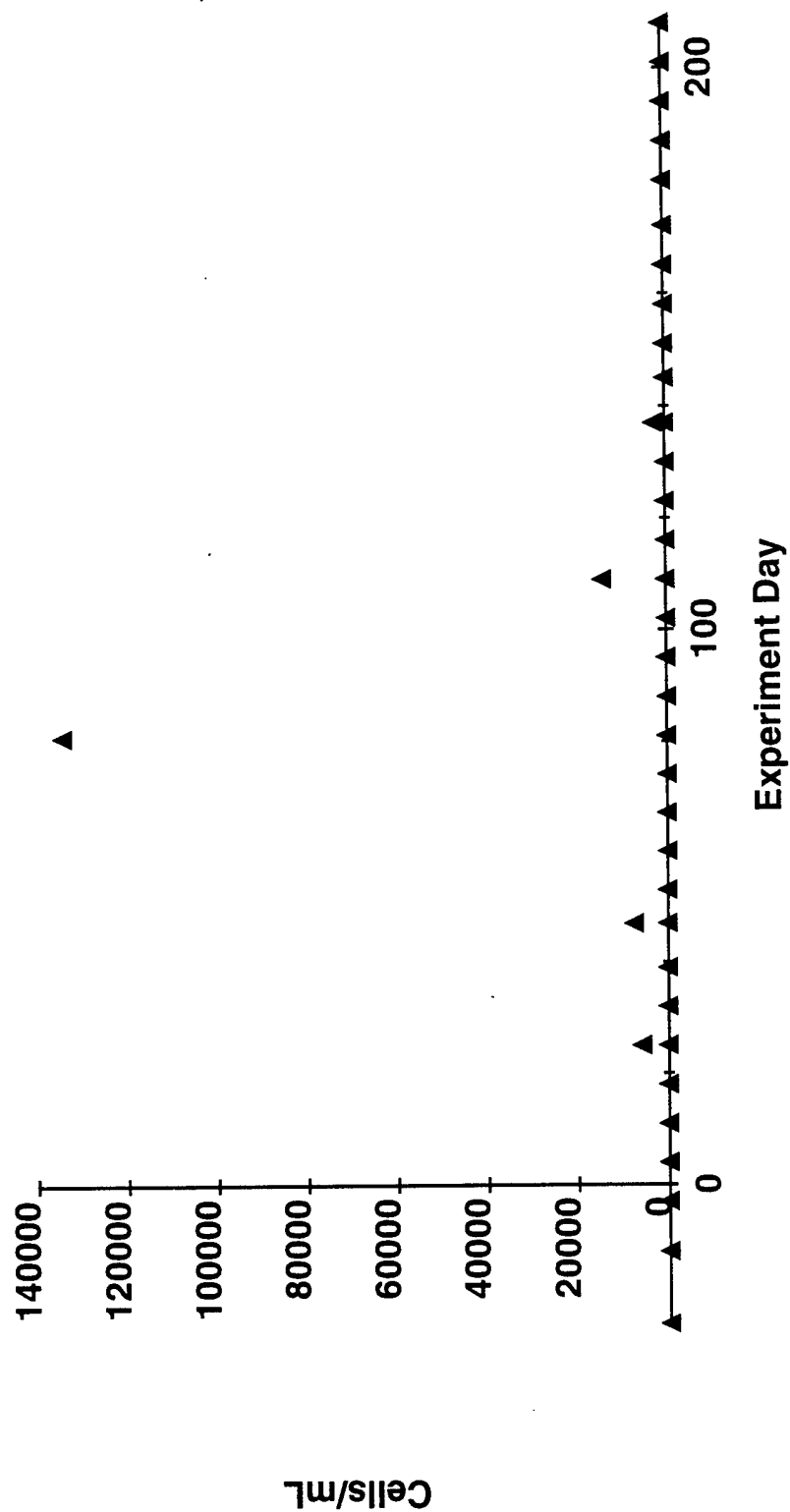
Figure 63c. Phytoplankton - Cyanophytes
Oscillatoria - Low Dose
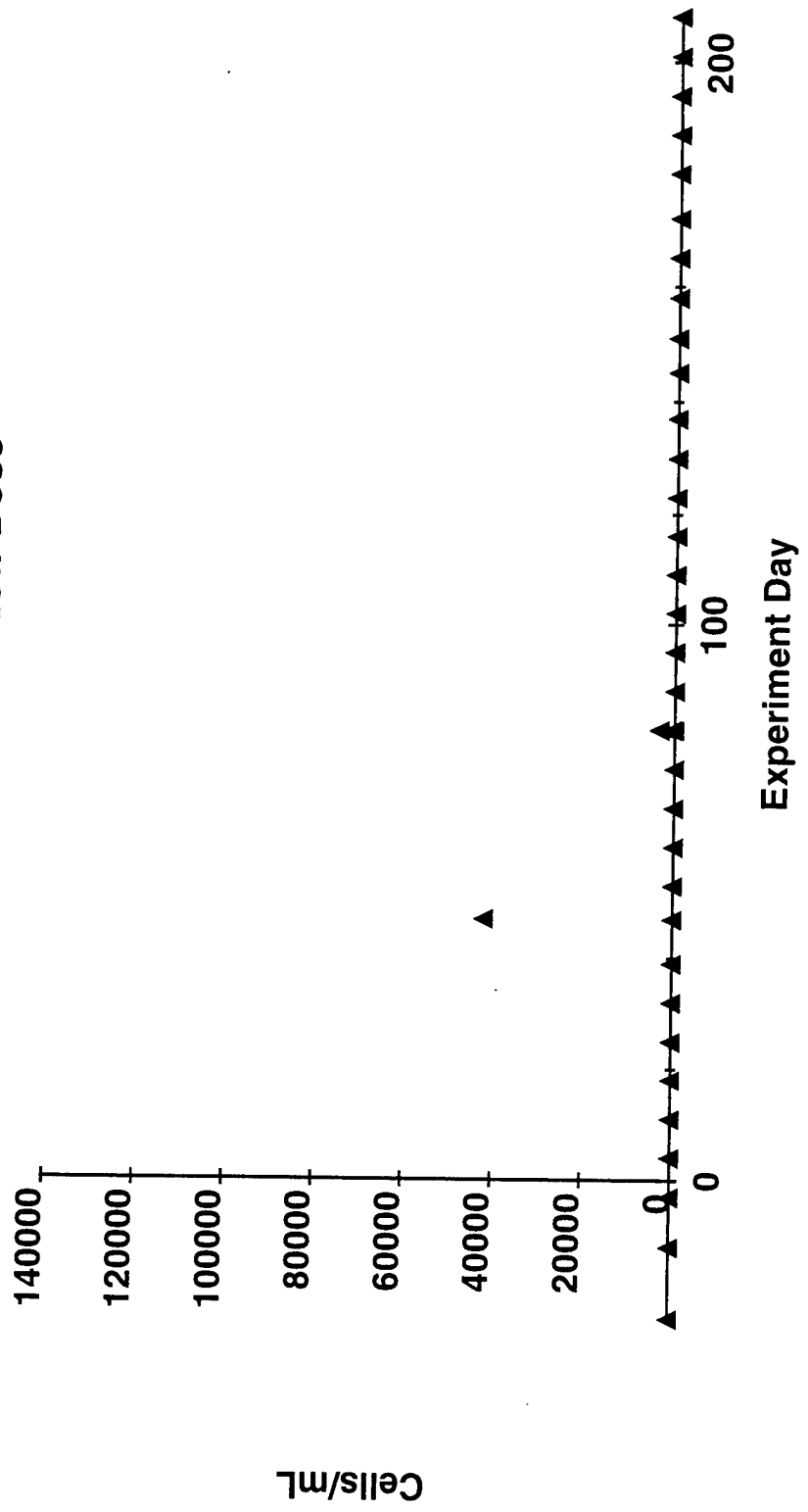
Figure 63d. Phytoplankton - Cyanophytes
Oscillatoria - Undosed
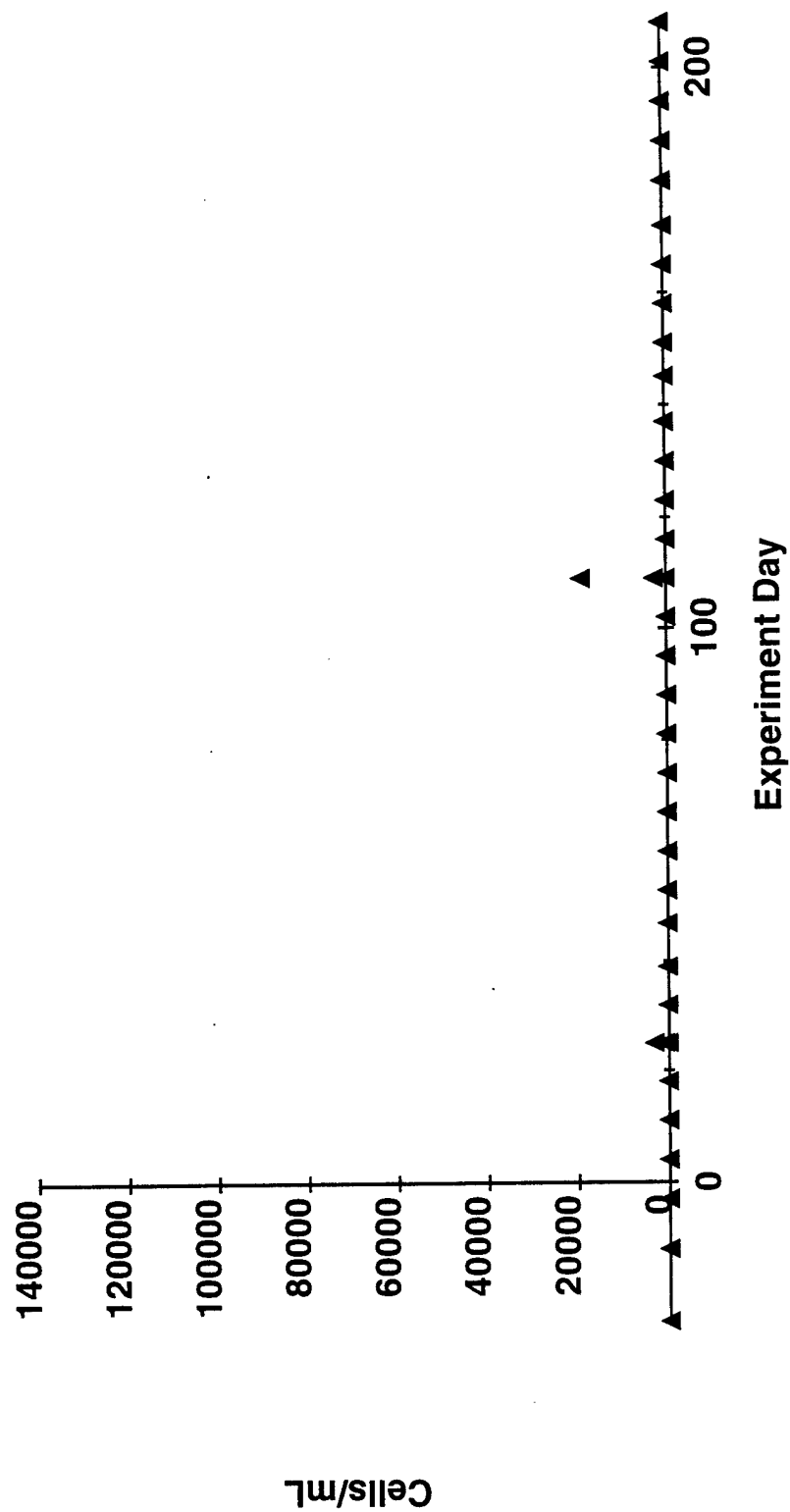
Figure 64a. Phytoplankton - Diatoms
Centric - High Dose
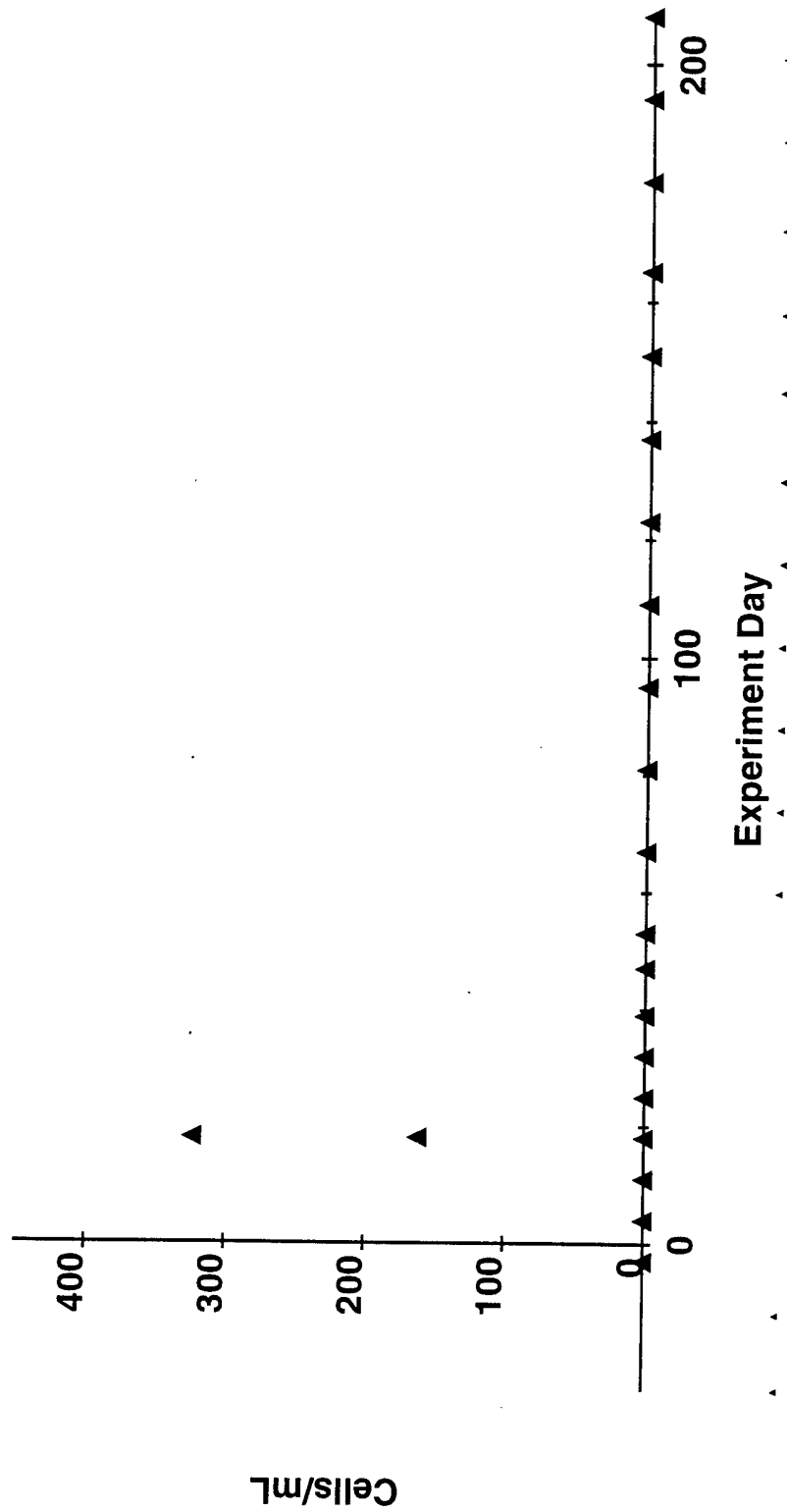
Figure 64b. Phytoplankton - Diatoms
Centric - Mid-dose
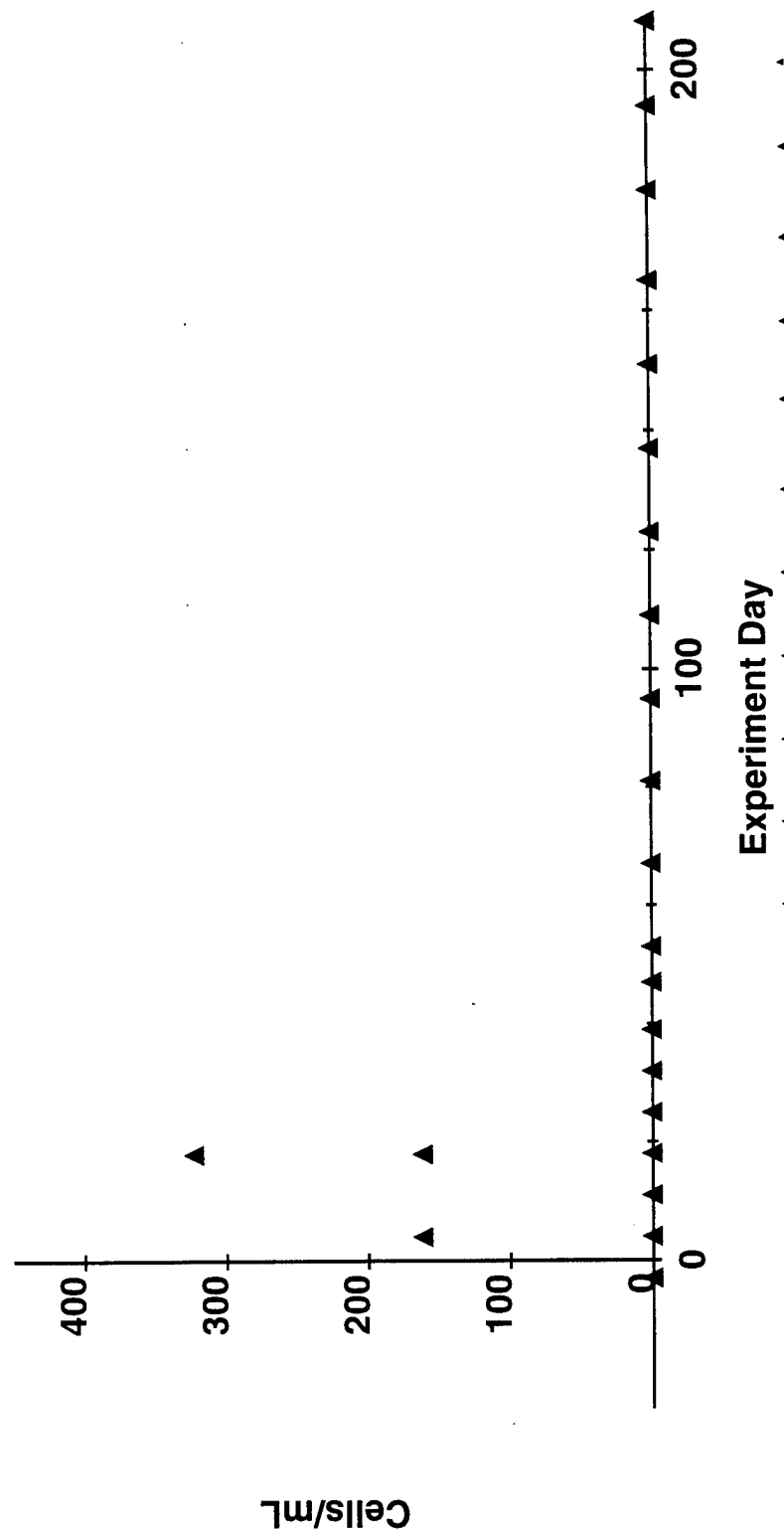
Figure 64c. Phytoplankton - Diatoms
Centric - Low Dose
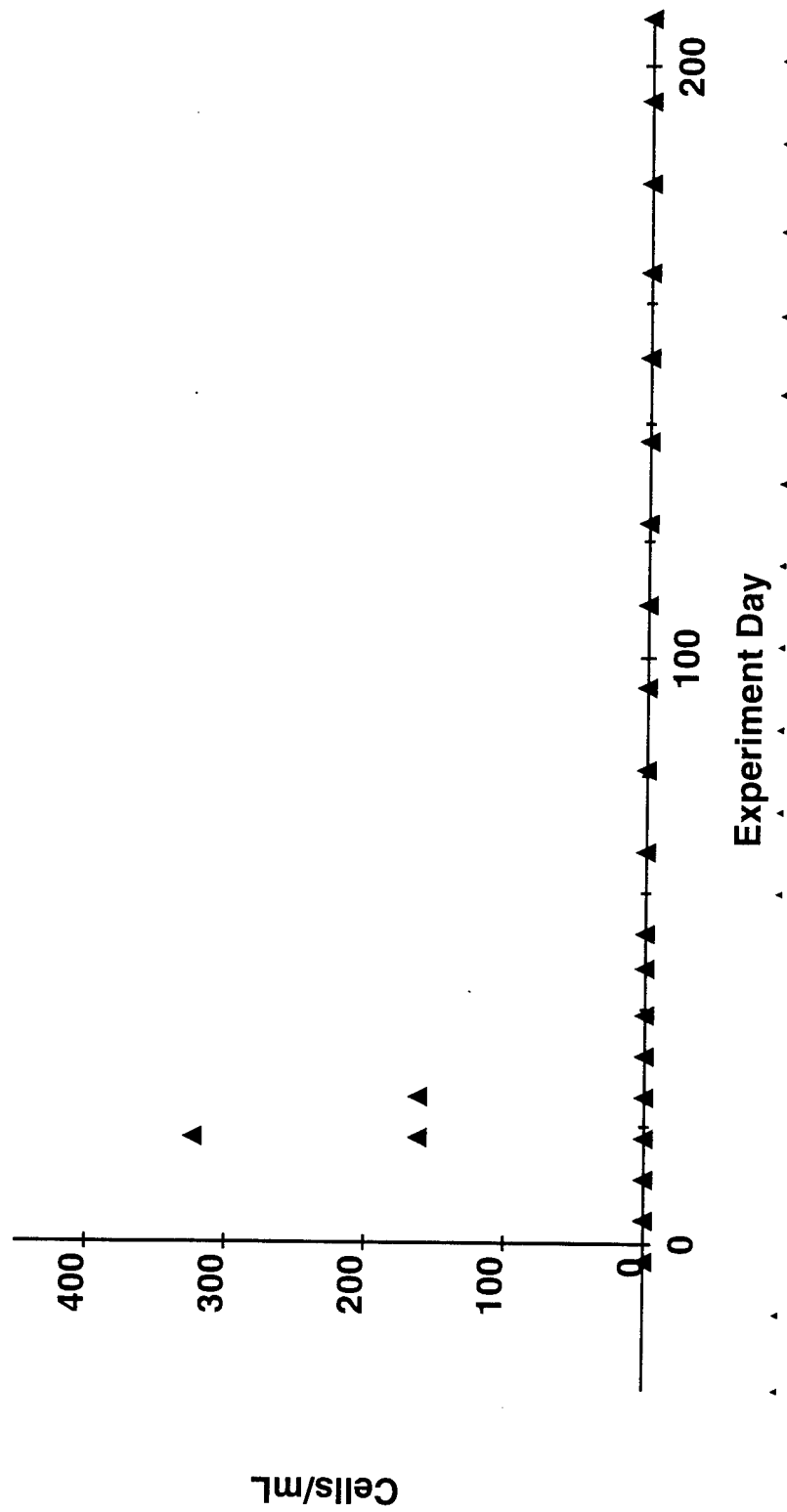
Figure 64d. Phytoplankton - Diatoms
Centric - Undosed
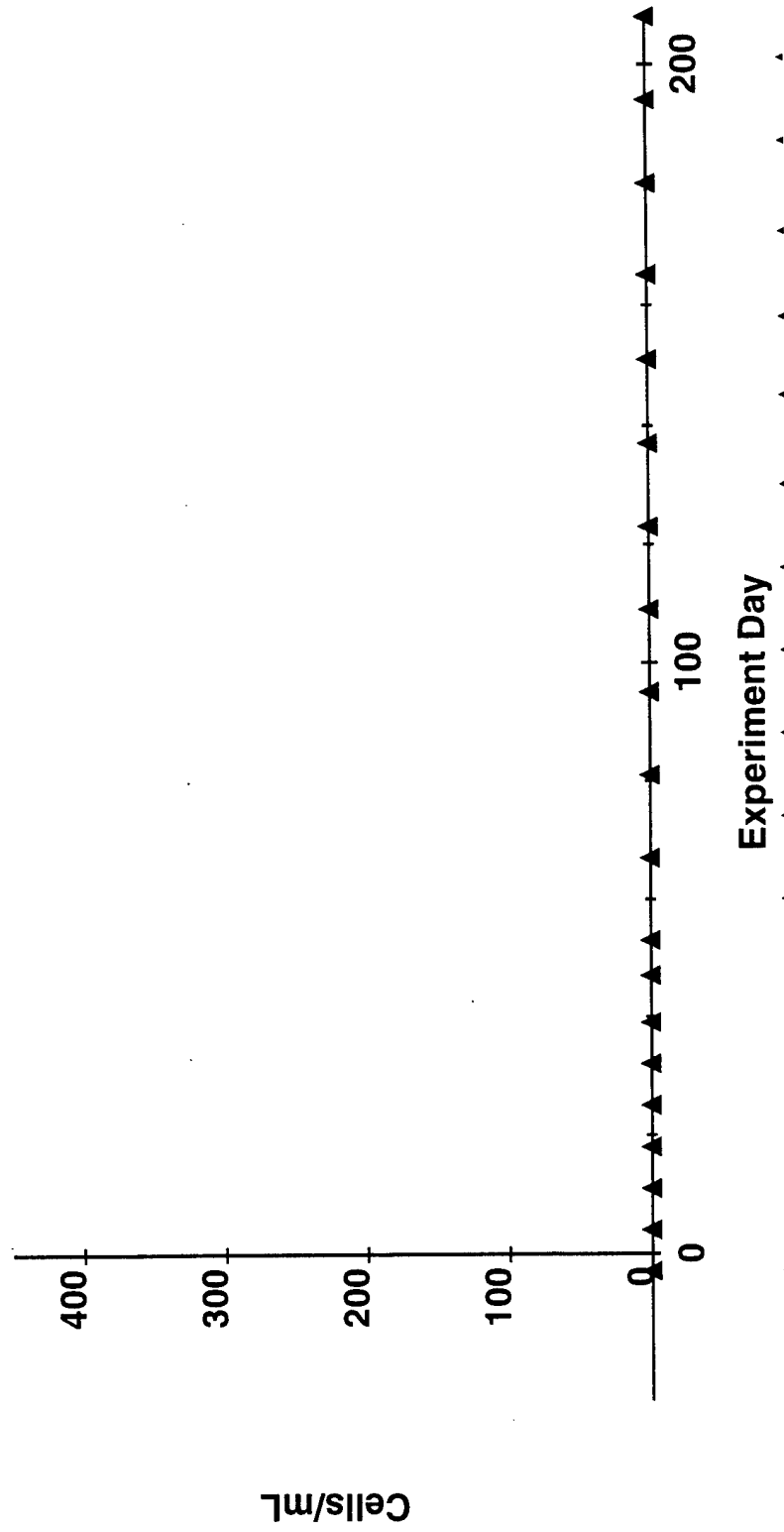
Figure 65a. Phytoplankton - Diatoms
Pennate - High Dose
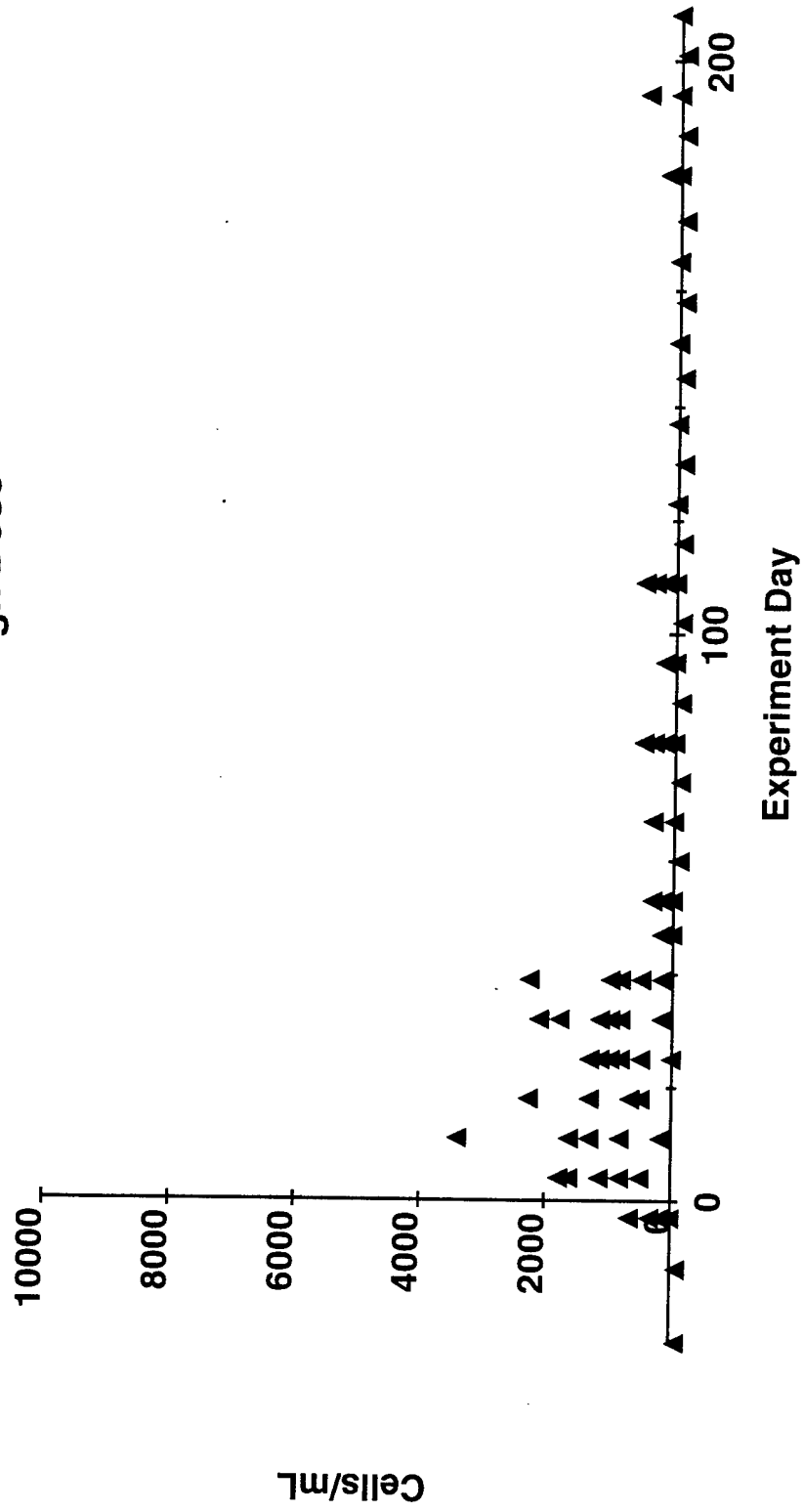
Figure 65b. Phytoplankton - Diatoms
Pennate - Mid-dose
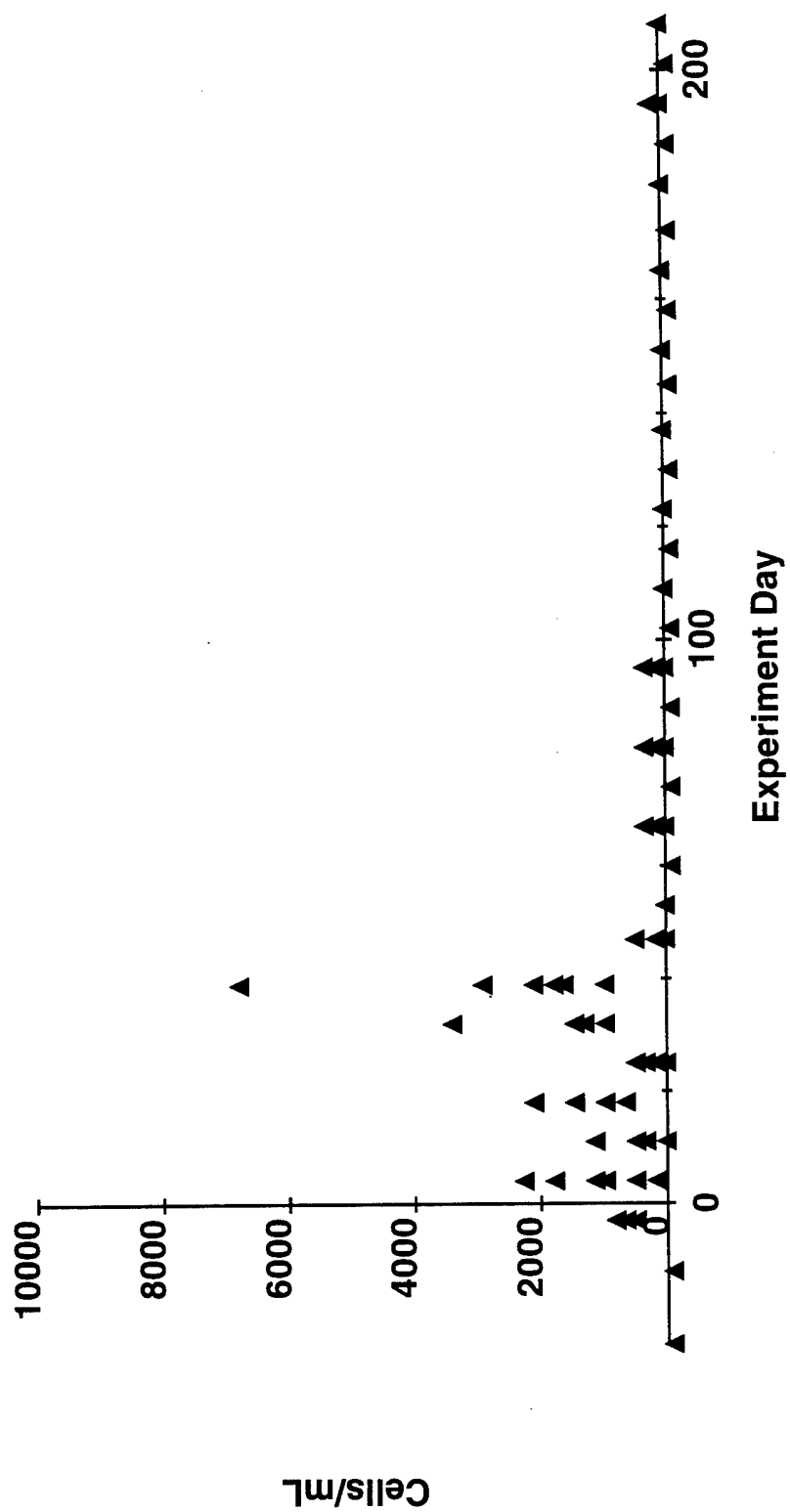
Figure 65c. Phytoplankton - Diatoms
Pennate - Low Dose
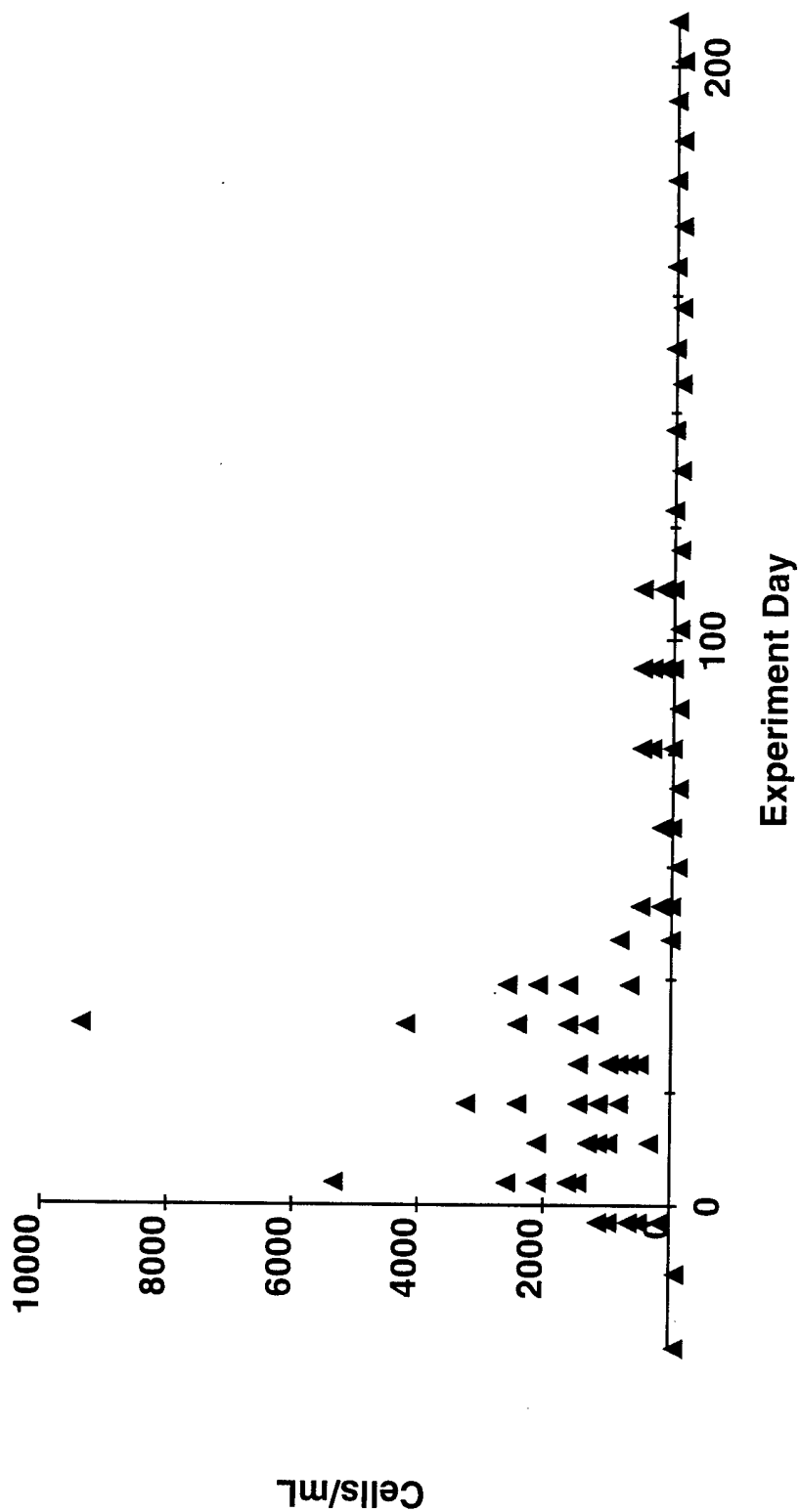
Figure 65d. Phytoplankton - Diatoms
Pennate - Undosed
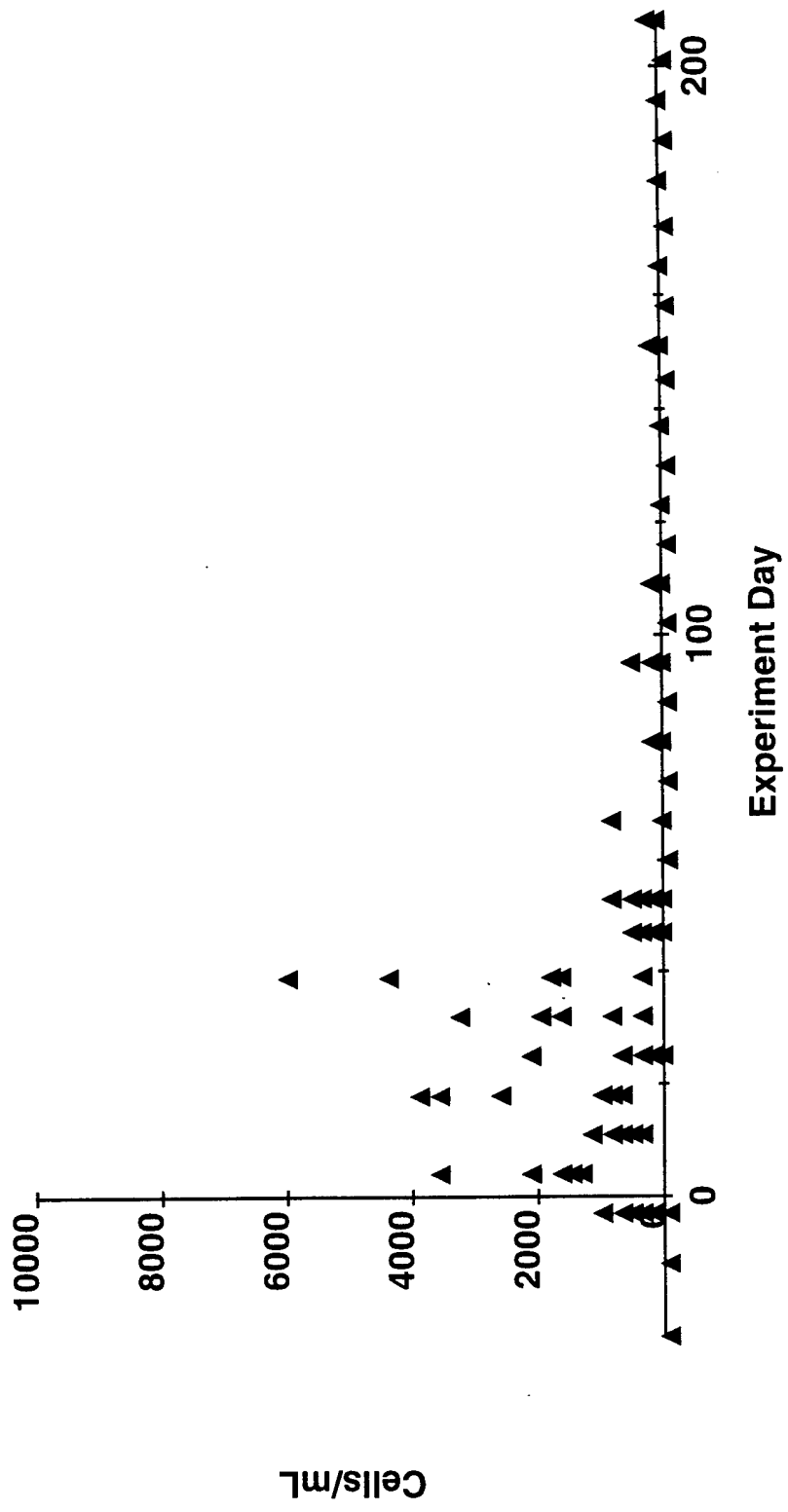
Figure 66a. Phytoplankton - Flagellates
Euglenoids - High Dose
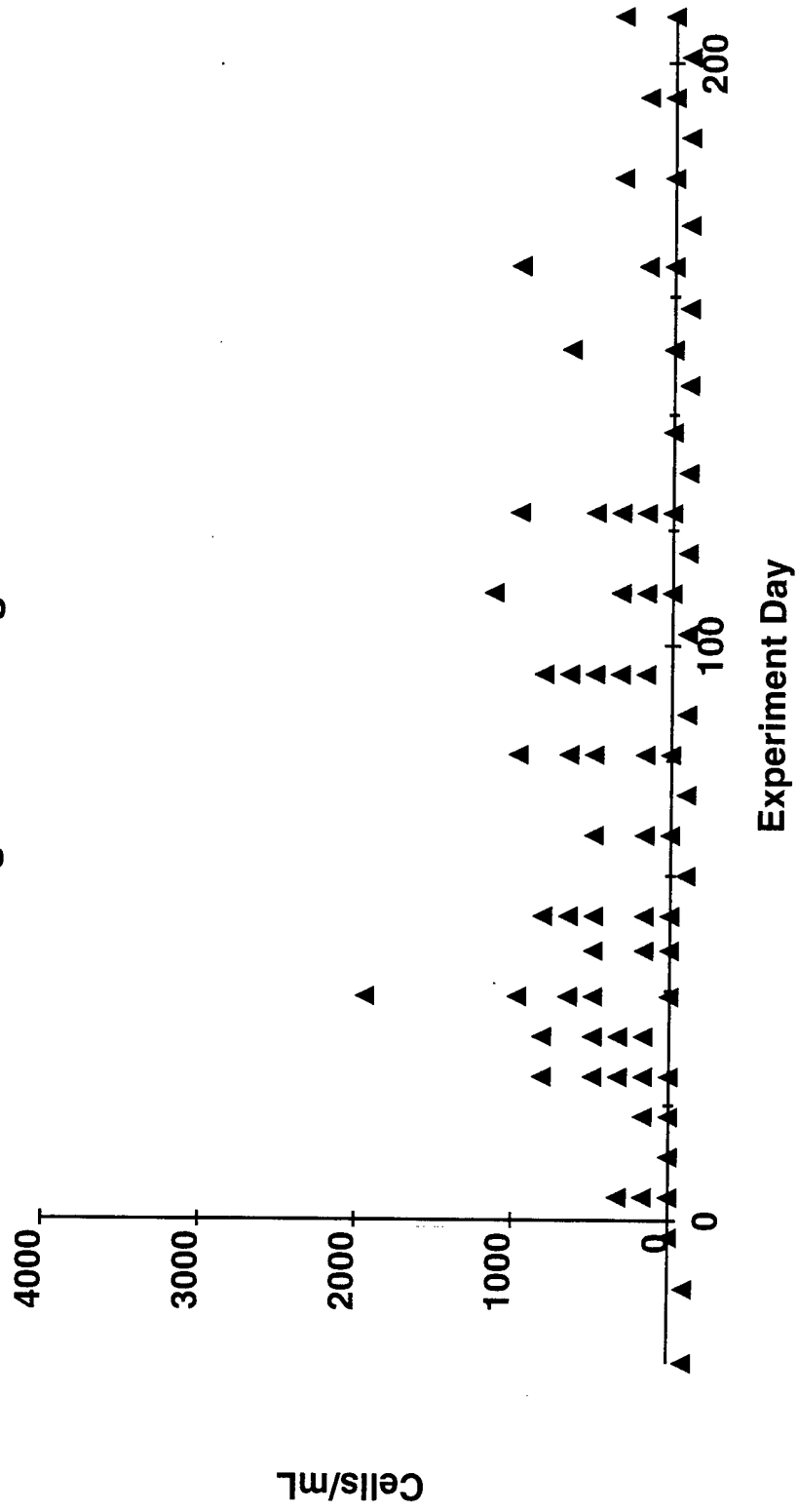
Figure 66b. Phytoplankton - Flagellates
Euglenoids - Mid-dose
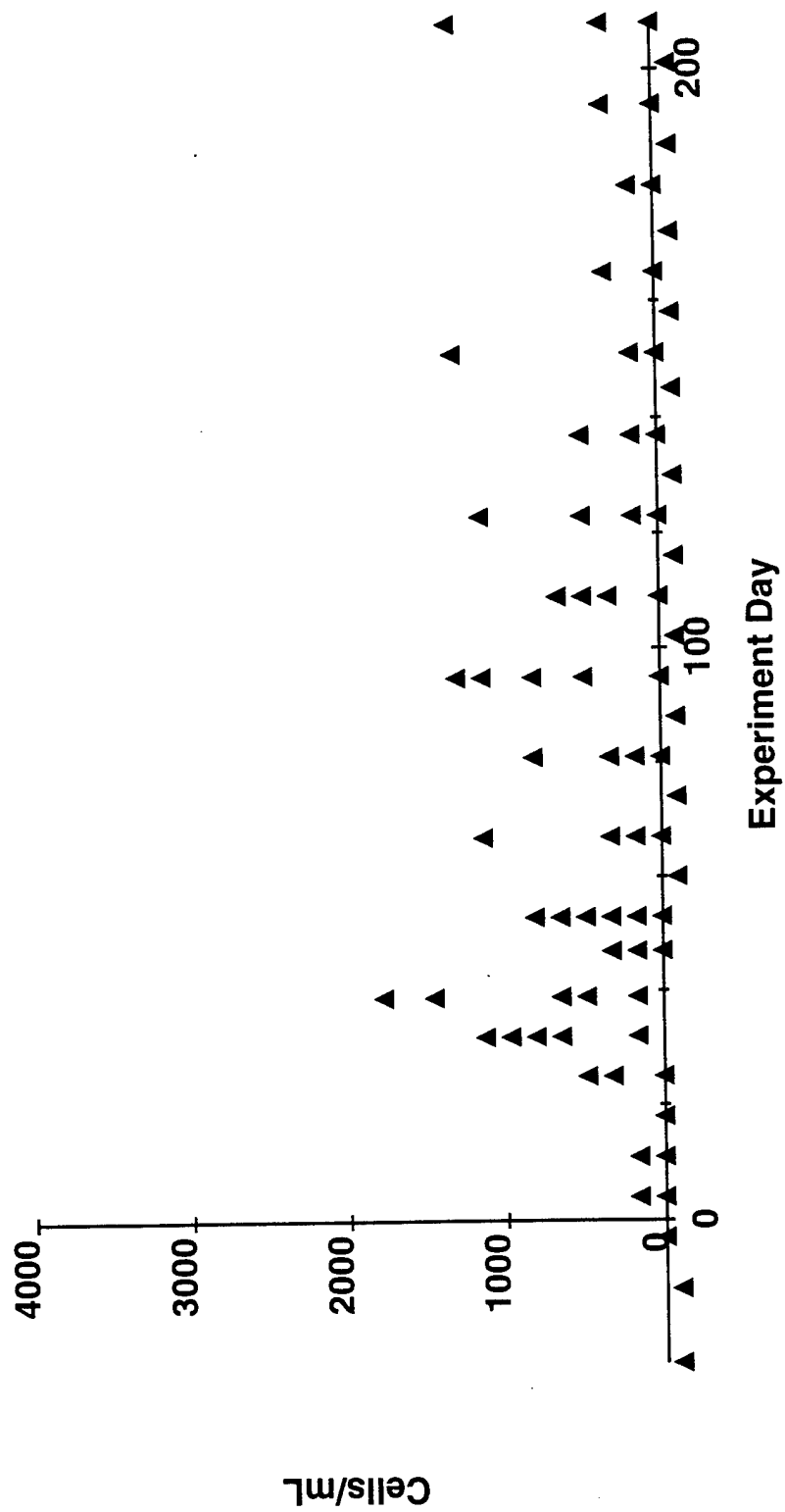
Figure 66c. Phytoplankton - Flagellates
Euglenoids - Low Dose
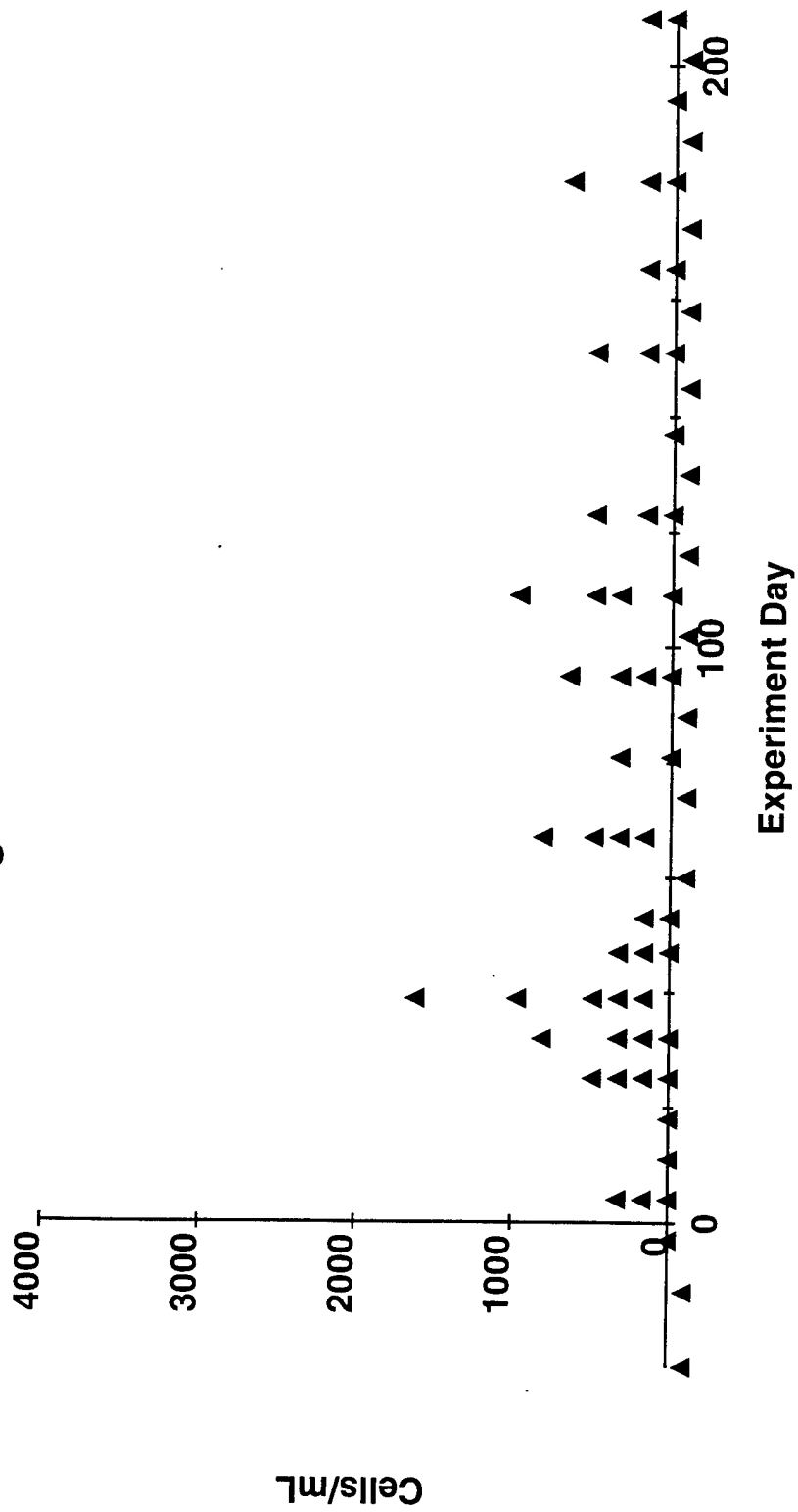
Figure 66d. Phytoplankton - Flagellates
Euglenoids - Undosed
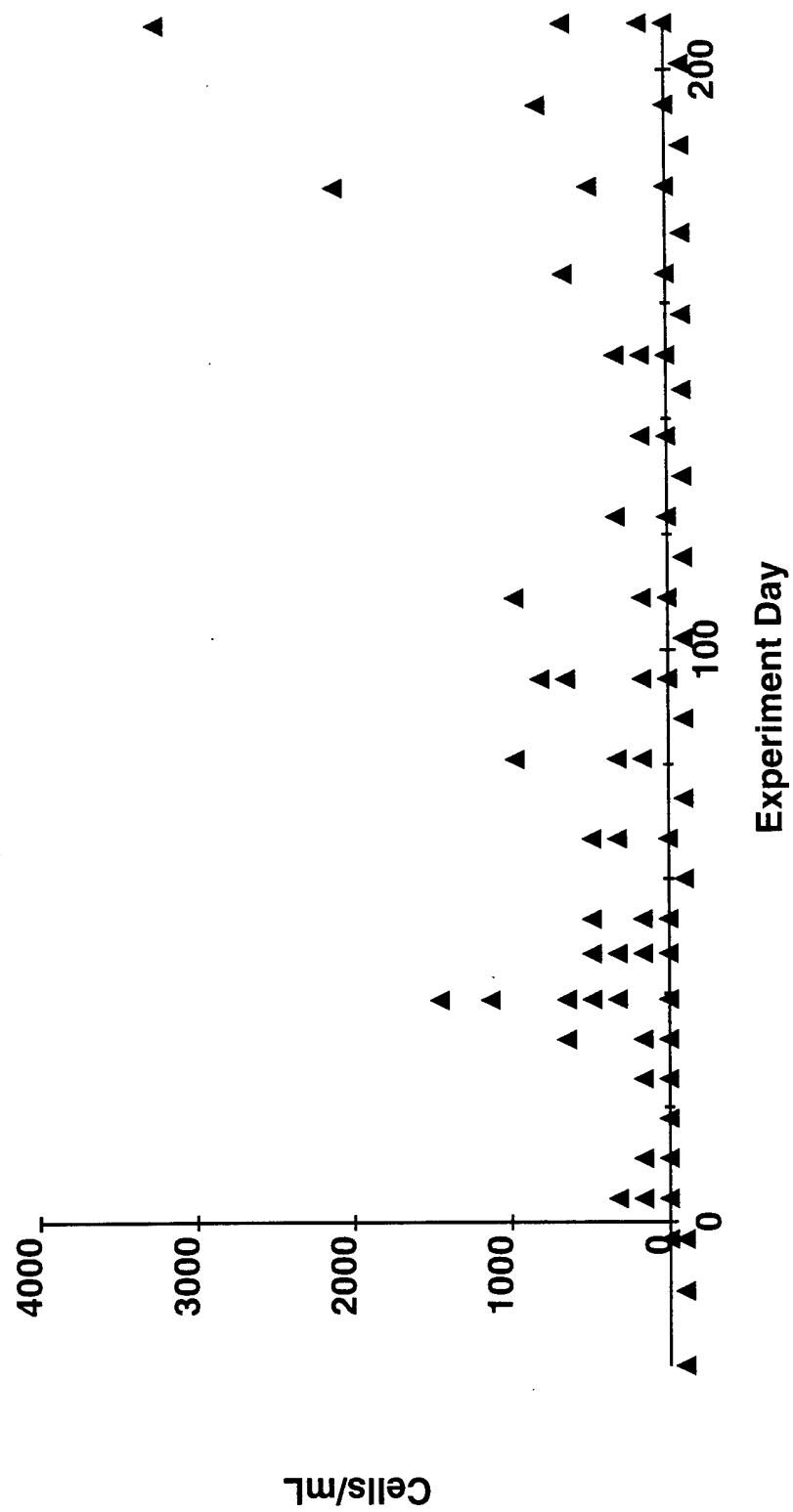
Figure 67a. Phytoplankton - Flagellates
Heterotrophs - High Dose
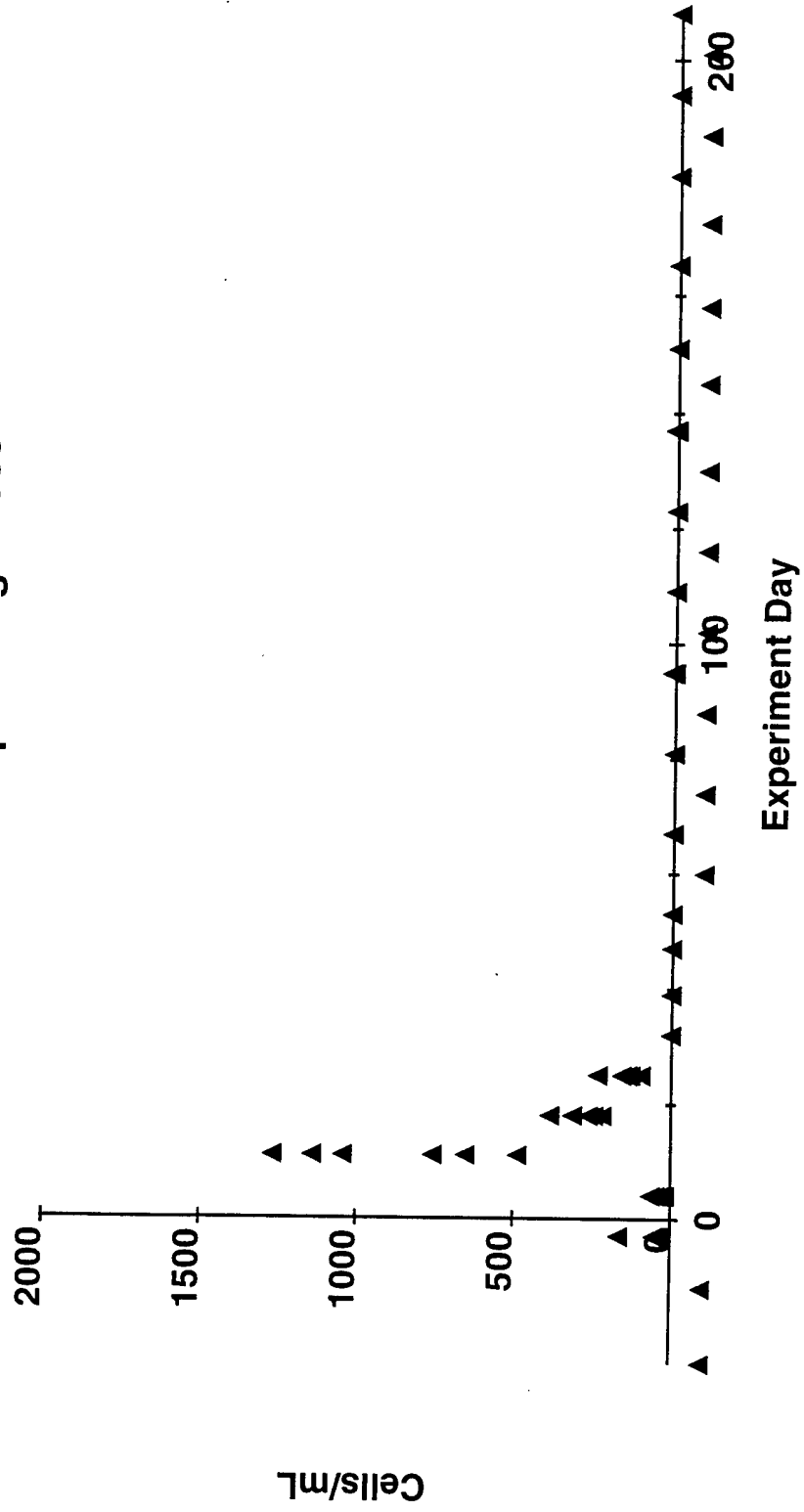
Figure 67b. Phytoplankton - Flagellates
Heterotrophs - Mid-dose
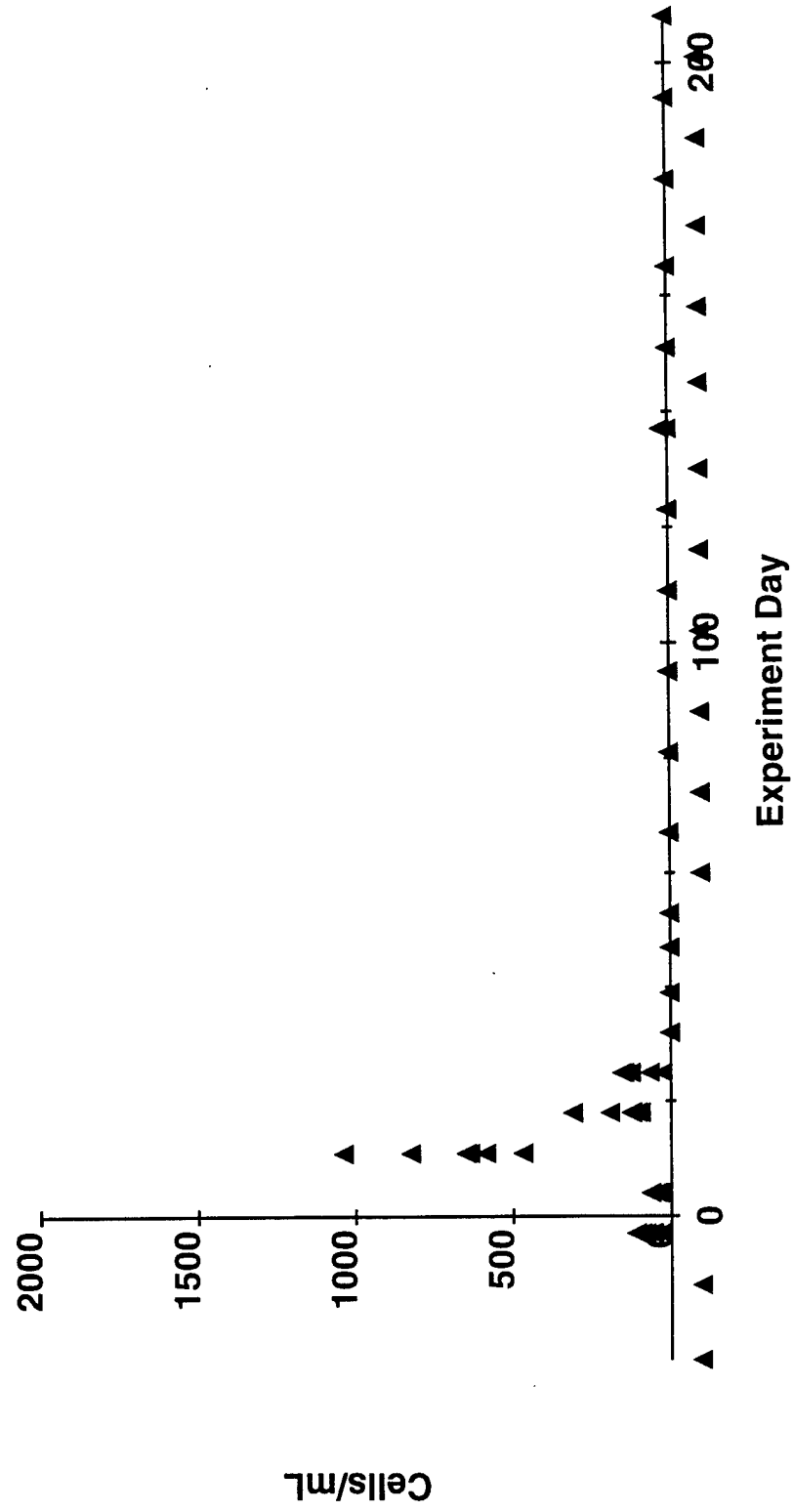
Figure 67c. Phytoplankton - Flagellates
Heterotrophs - Low Dose
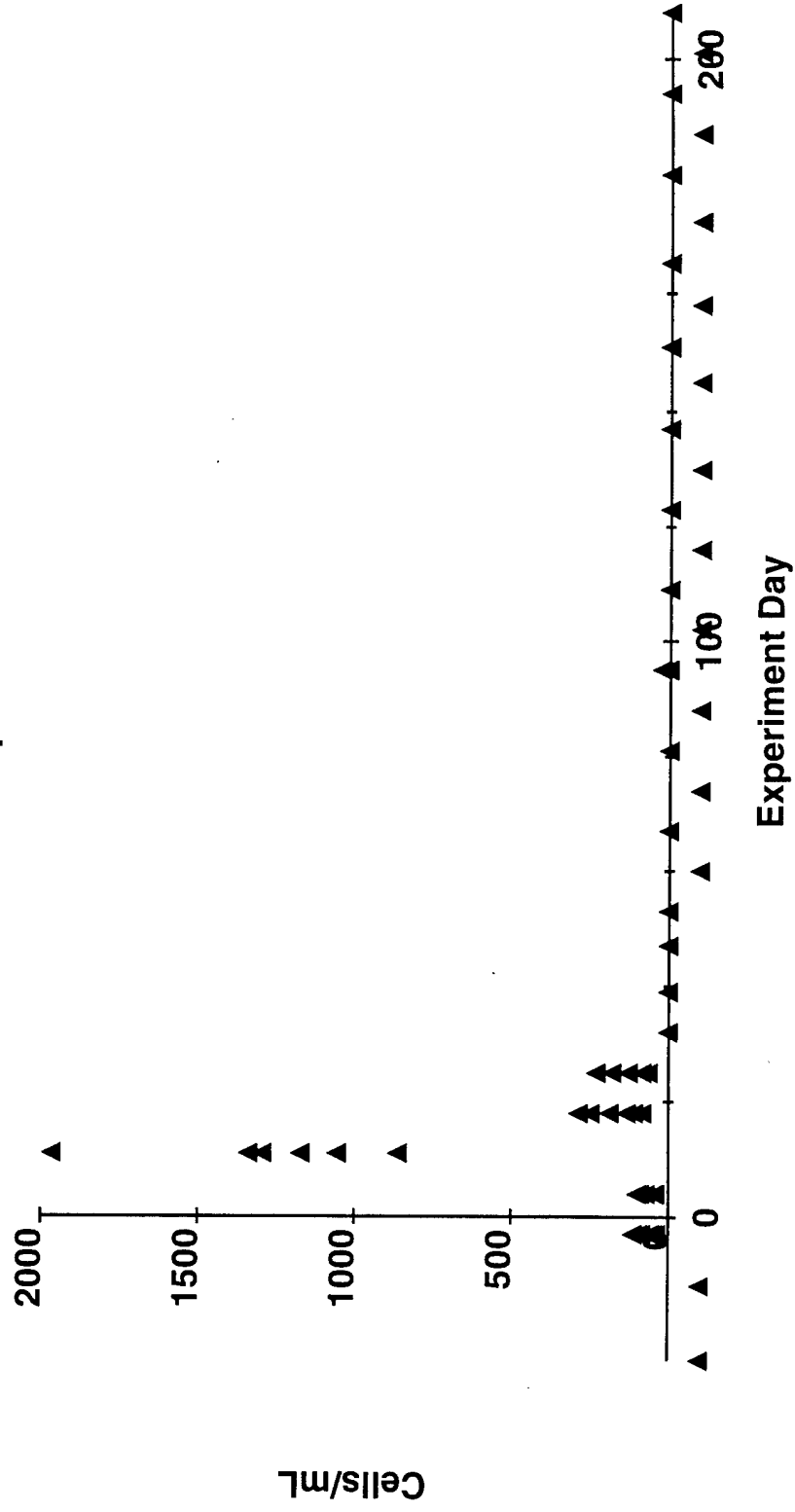
Figure 67d. Phytoplankton - Flagellates
Heterotrophs - Undosed
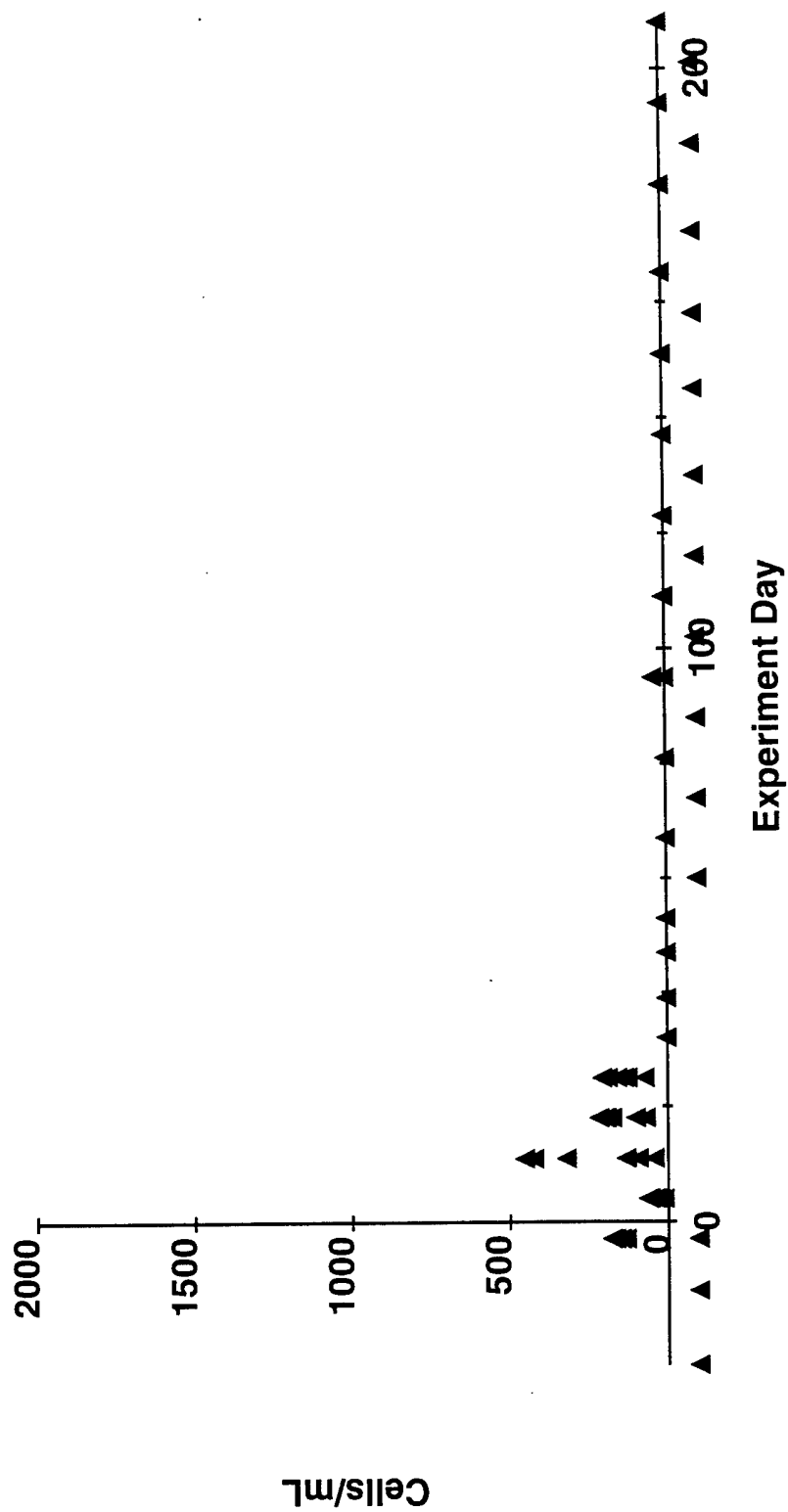
Figure 68a. Phytoplankton - Green Algae
Ankistrodesmus - High Dose
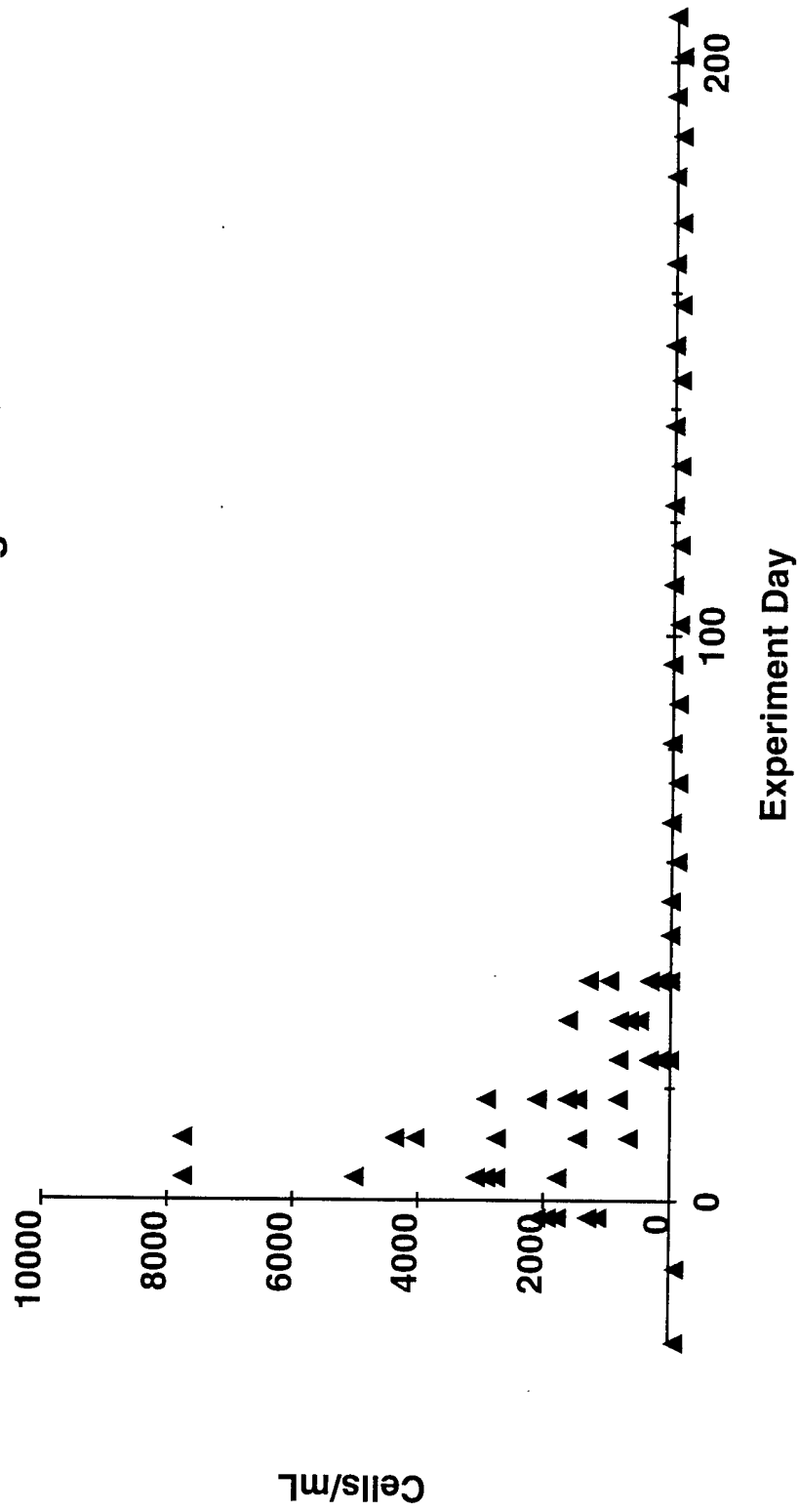
Figure 68b. Phytoplankton - Green Algae
Ankistrodesmus - Mid-dose
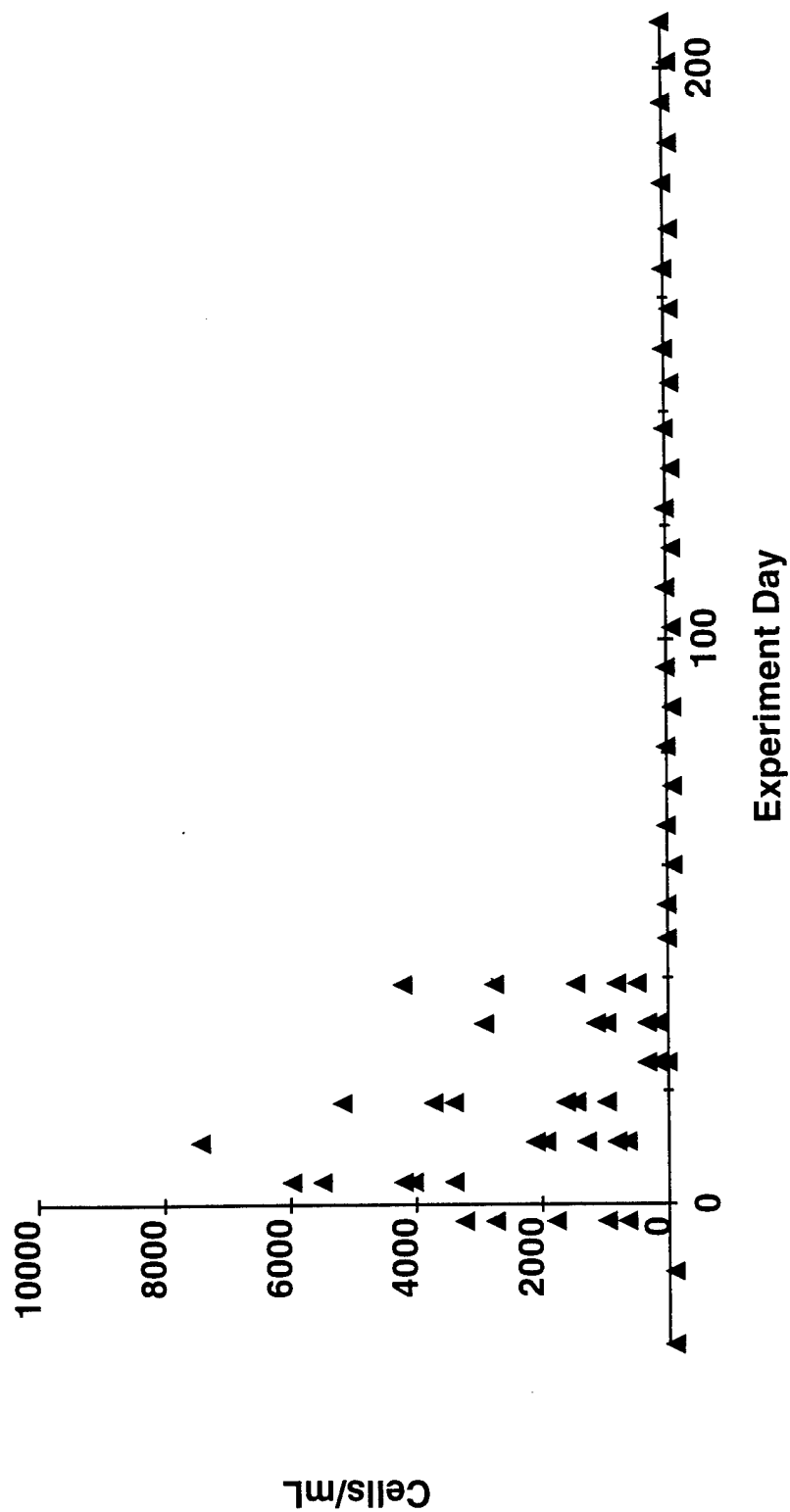
Figure 68c. Phytoplankton - Green Algae
Ankistrodesmus - Low Dose
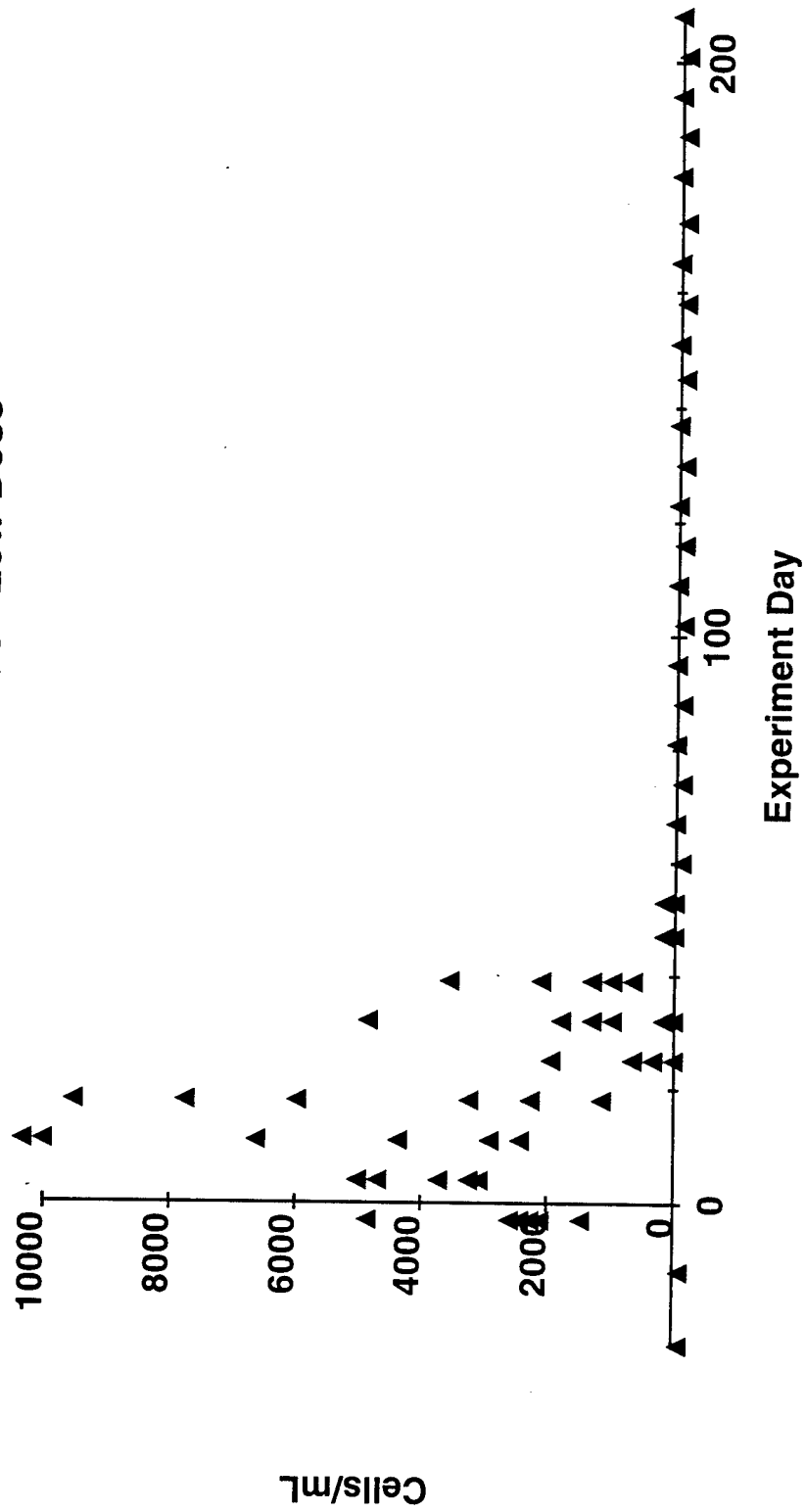
Figure 68d. Phytoplankton - Green Algae
Ankistrodesmus - Undosed
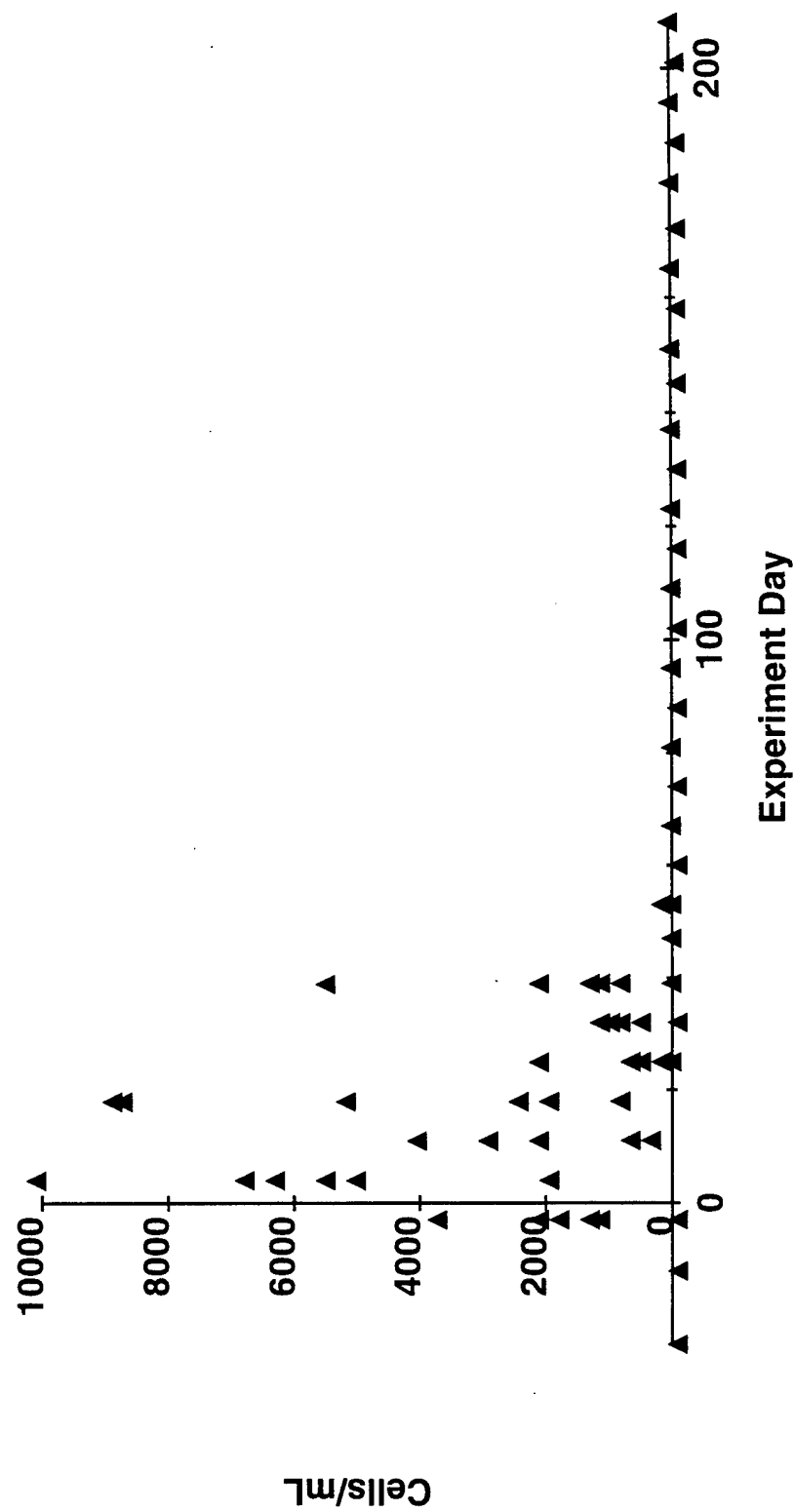
Figure 69a. Phytoplankton - Green Algae
Chlamydomonas - High Dose
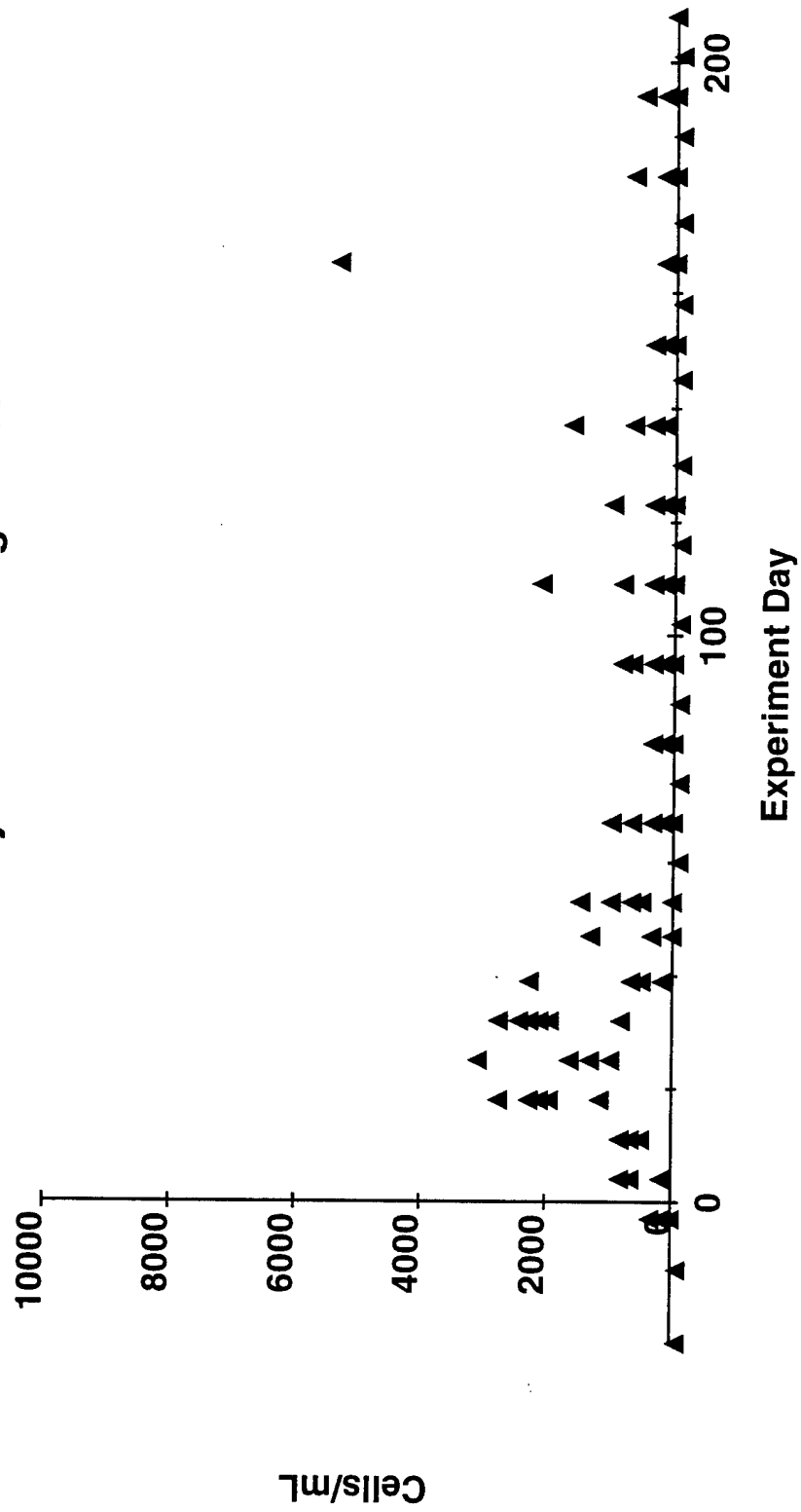
Figure 69b. Phytoplankton - Green Algae
Chlamydomonas - Mid-dose
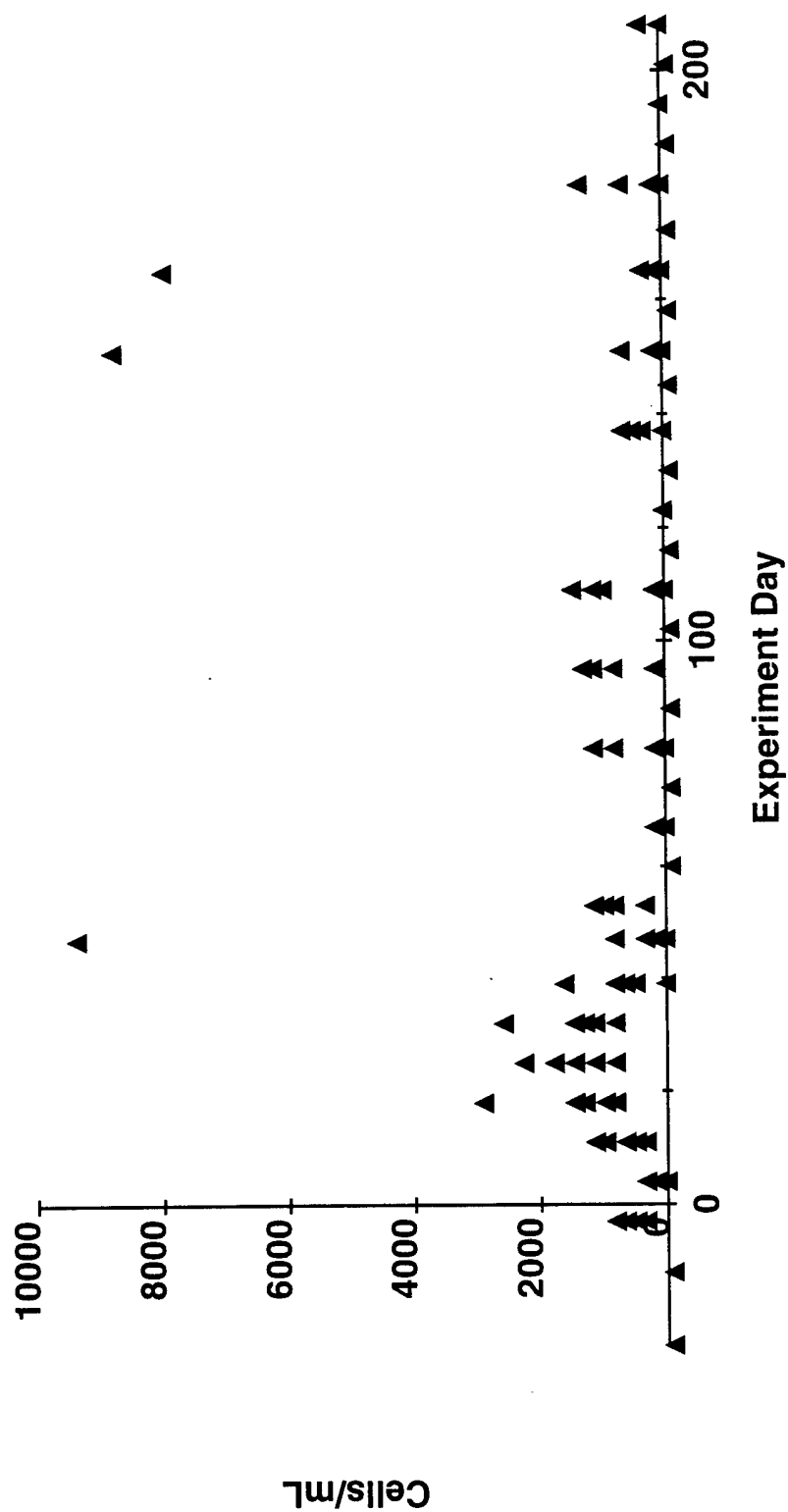
Figure 69c. Phytoplankton - Green Algae
Chlamydomonas - Low Dose
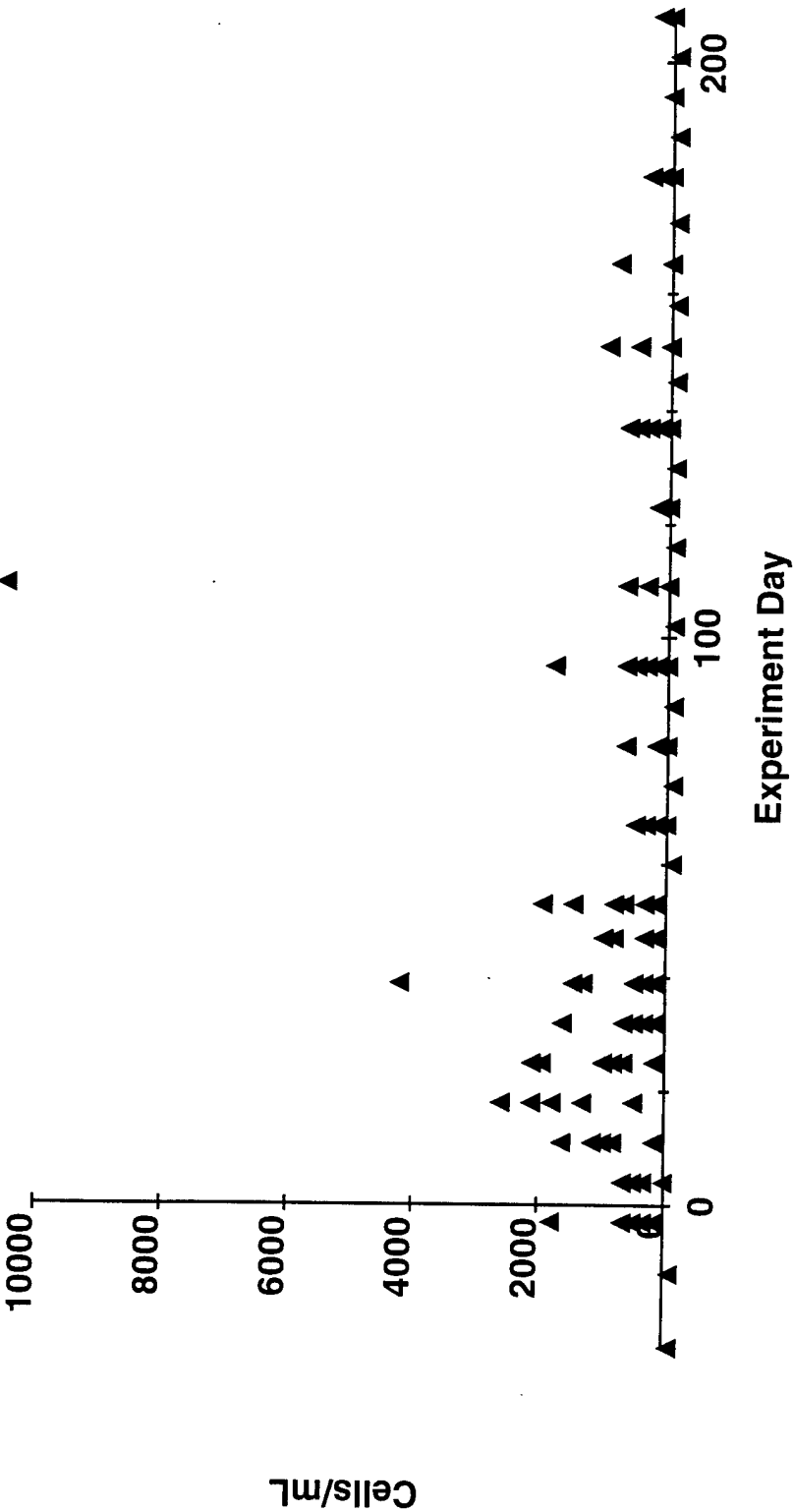
Figure 69d. Phytoplankton - Green Algae
Chlamydomonas - Undosed
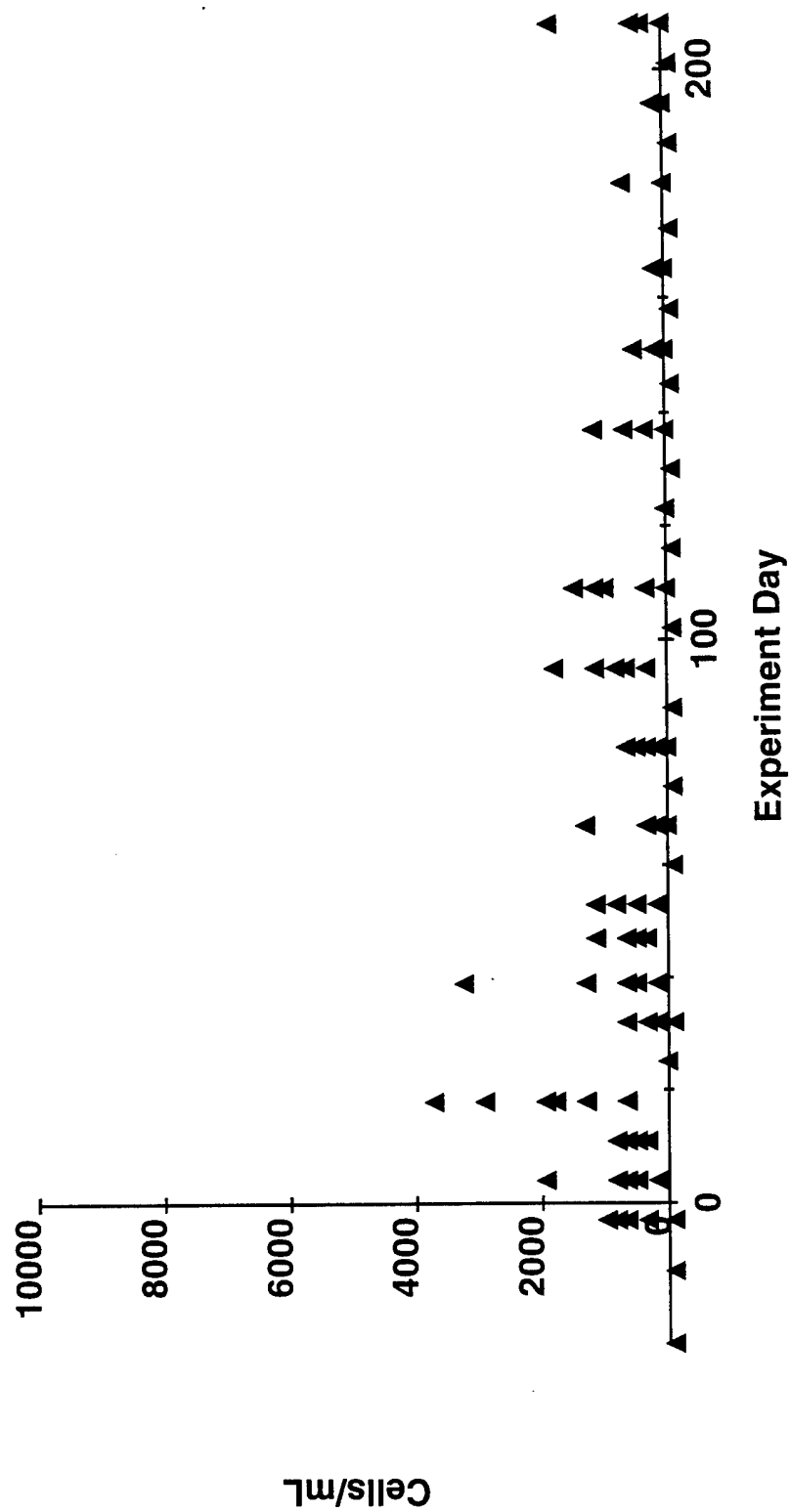
Figure 70a. Phytoplankton - Green Algae
Filamentous - High Dose
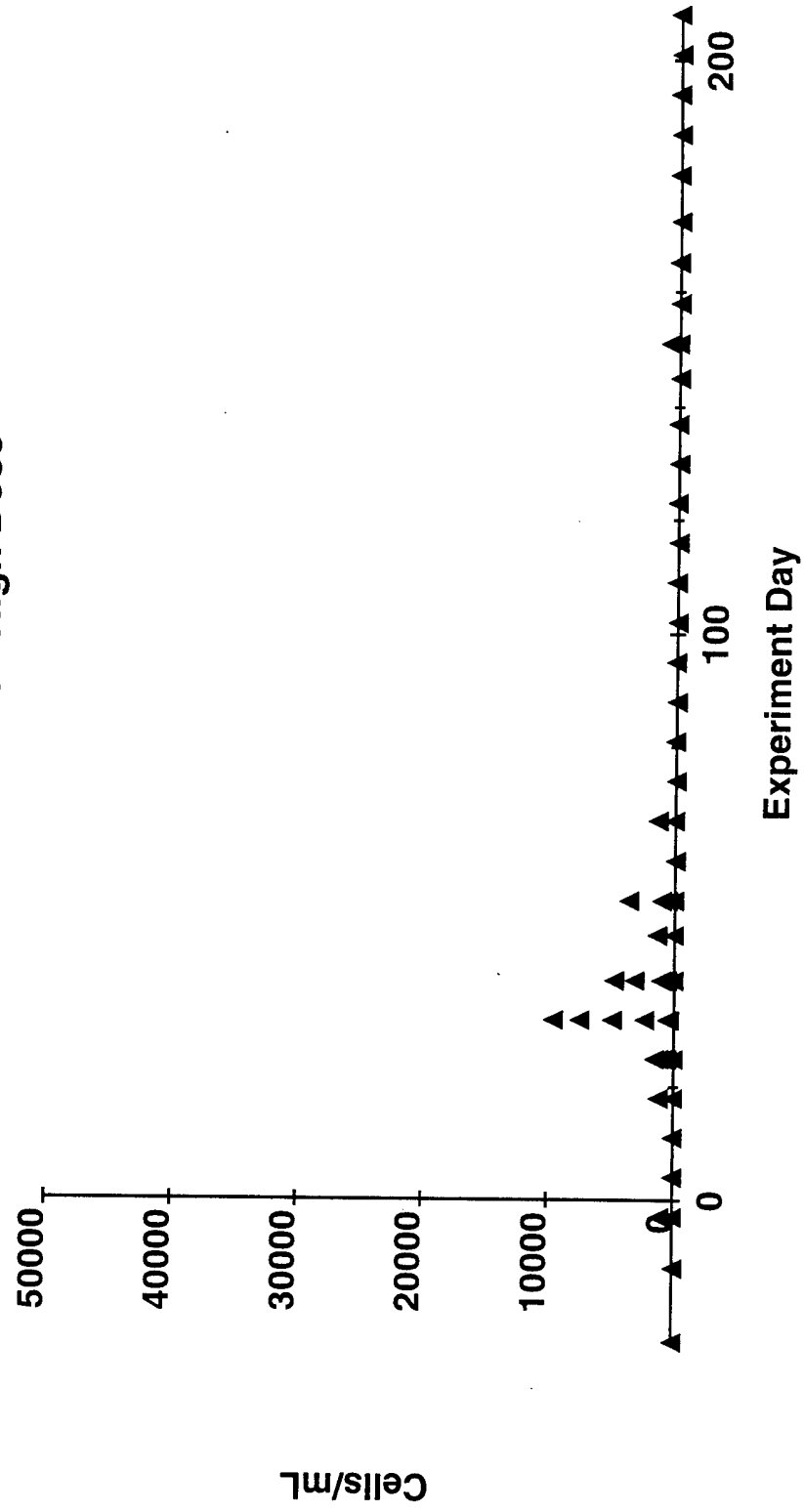
Figure 70b. Phytoplankton - Green Algae
Filamentous - Mid-dose
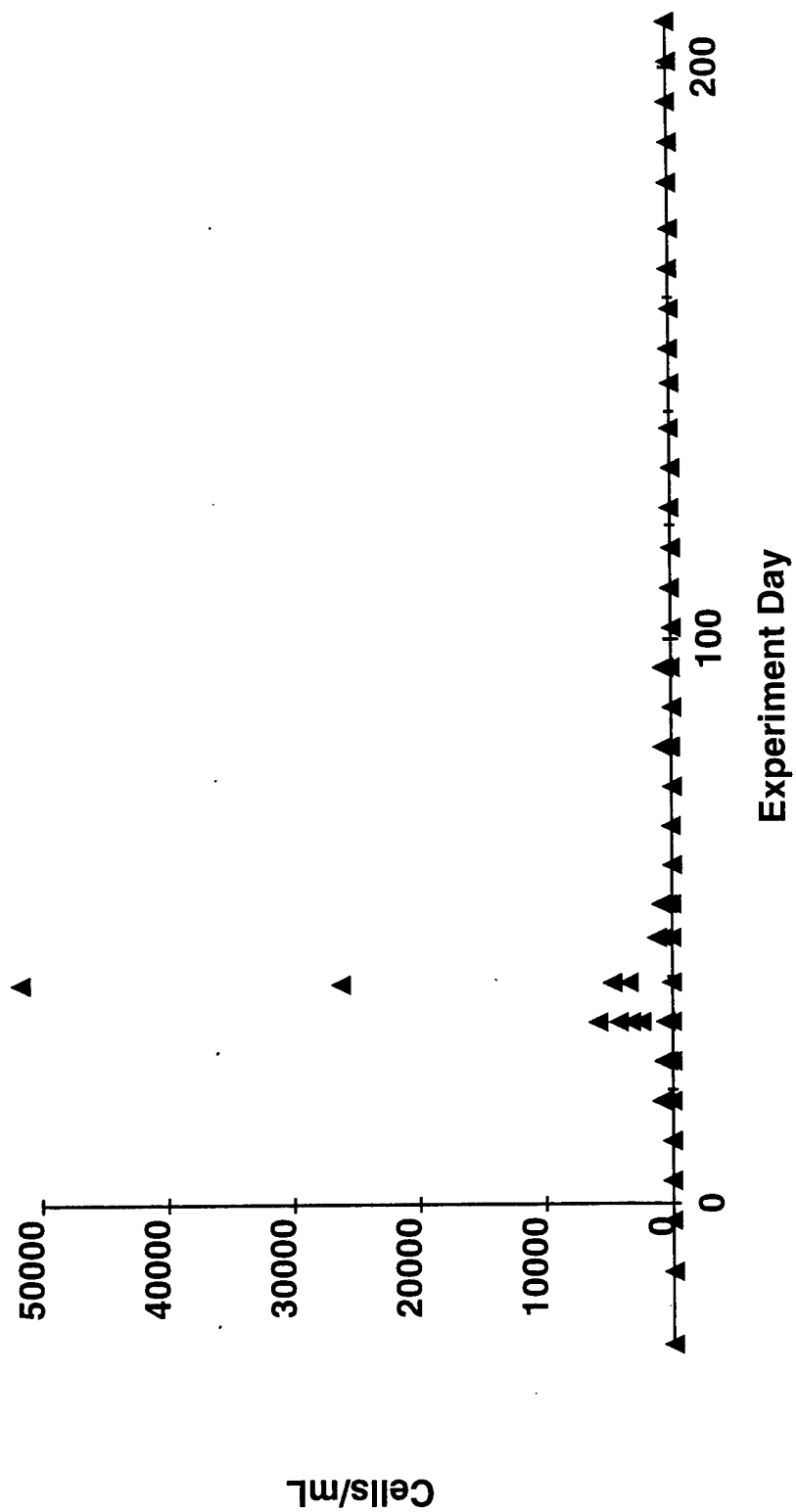
Figure 70c. Phytoplankton - Green Algae
Filamentous - Low Dose
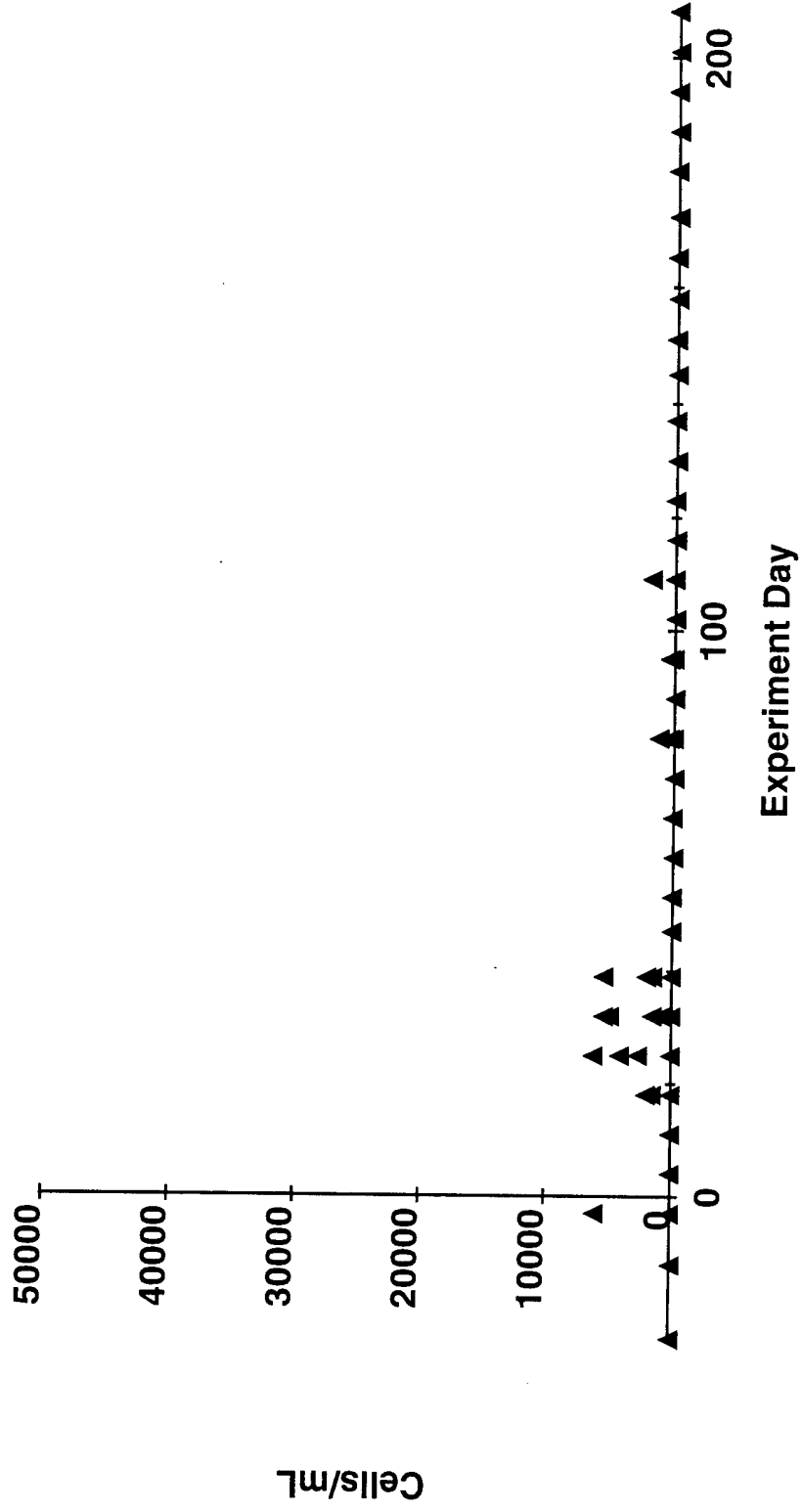
Figure 70d. Phytoplankton - Green Algae
Filamentous - Undosed
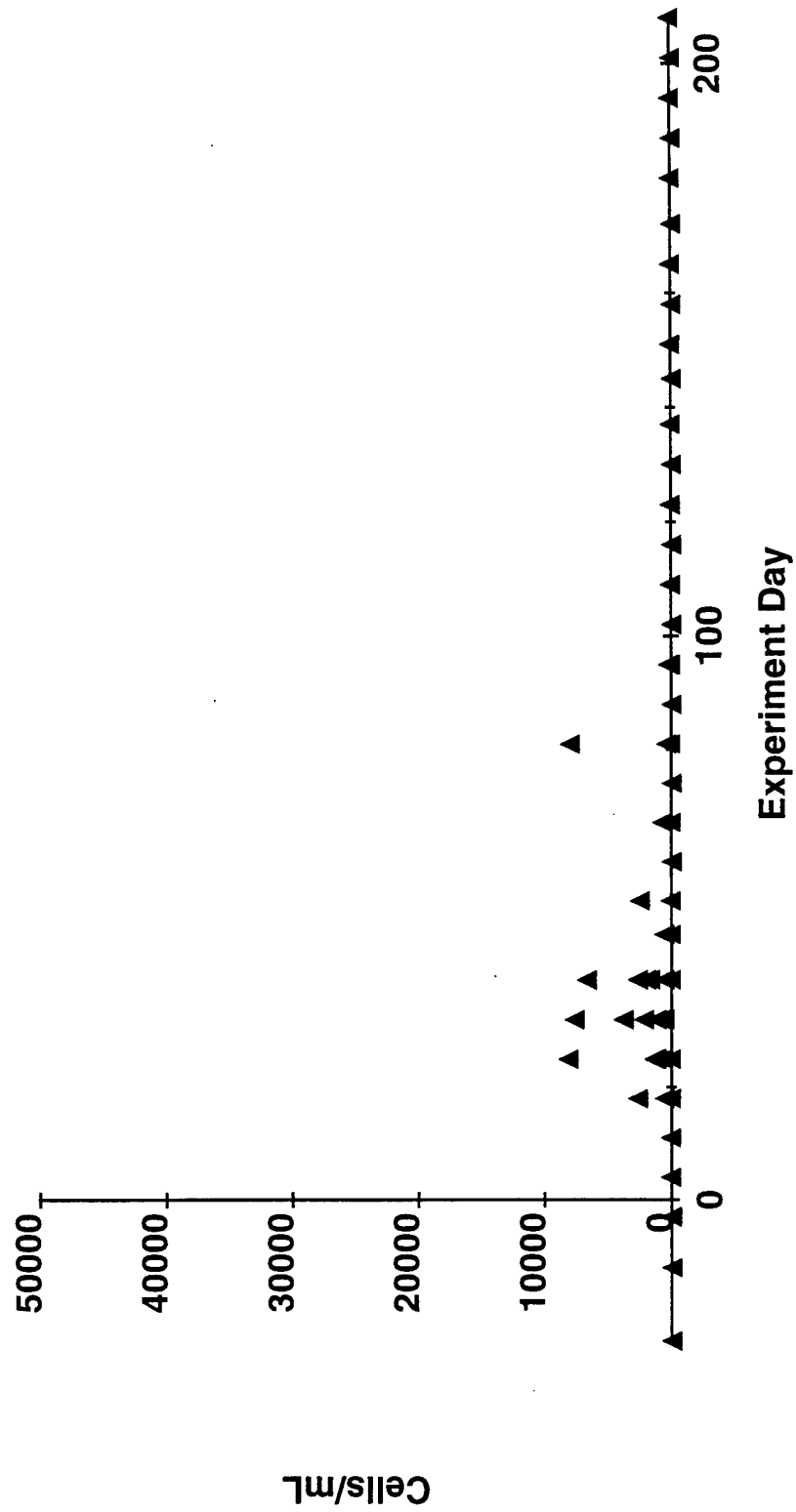
Figure 71a. Phytoplankton - Green Algae
Other - High Dose
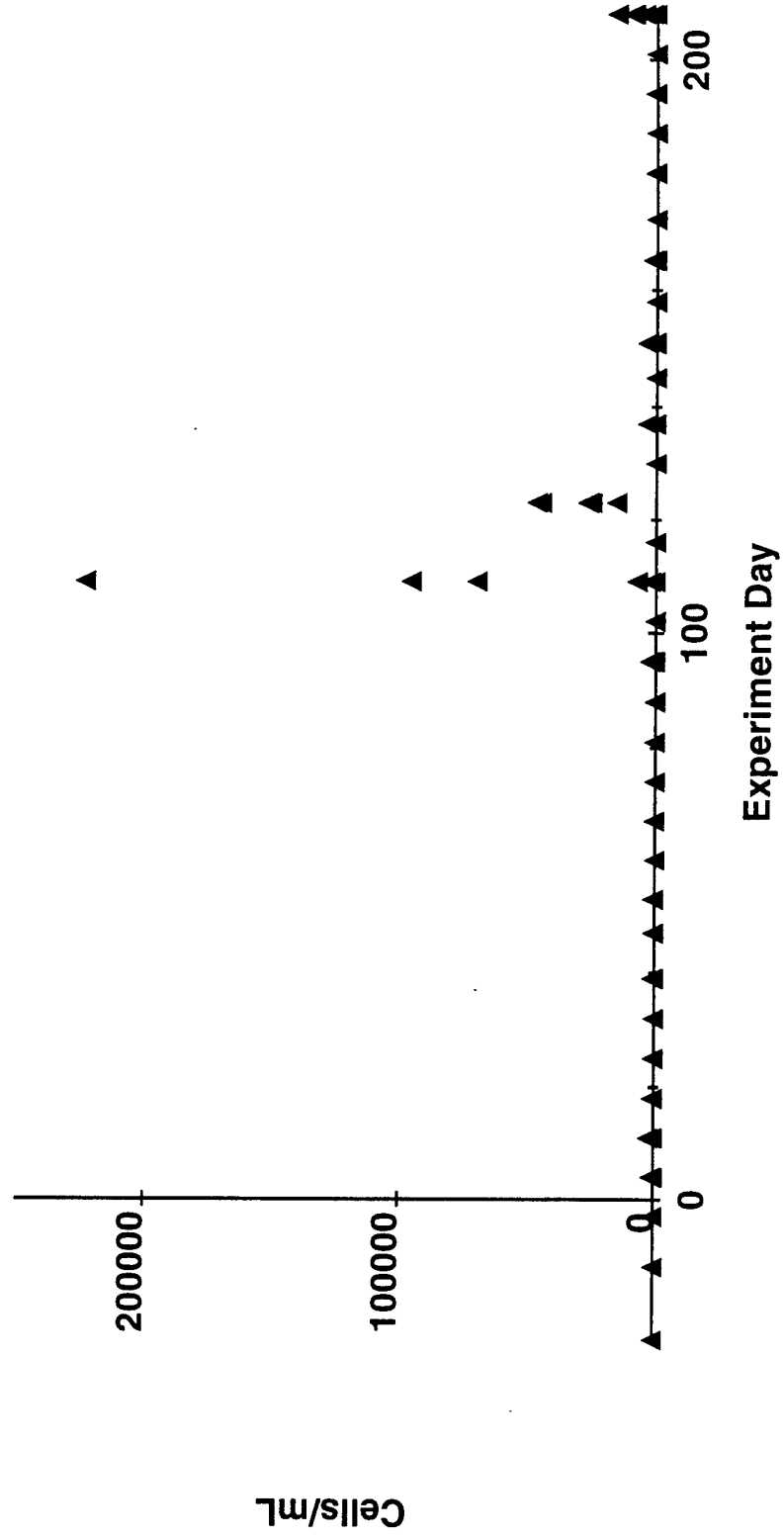
Figure 71b. Phytoplankton - Green Algae
Other - Mid-dose
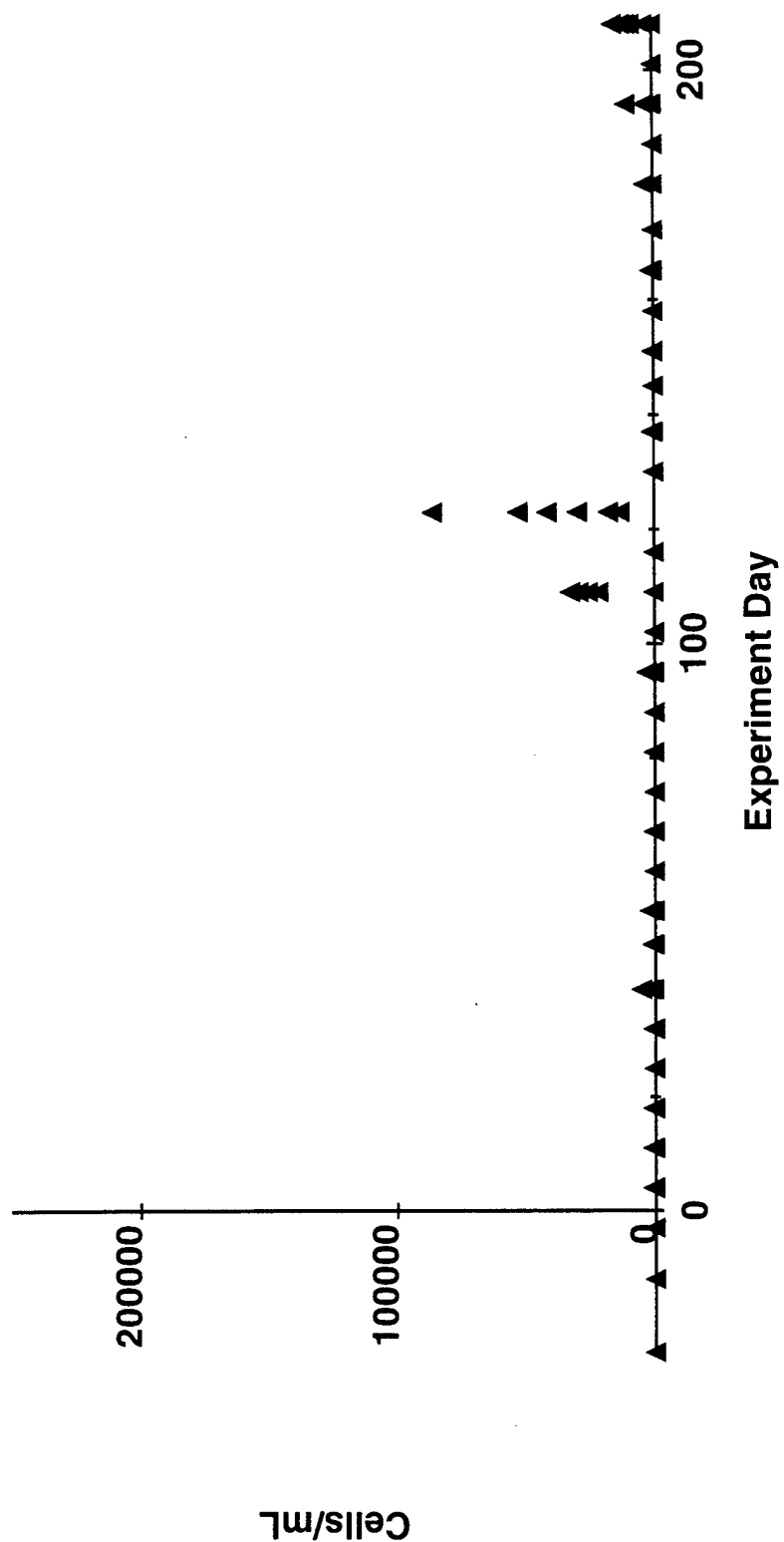
Figure 71c. Phytoplankton - Green Algae
Other - Low Dose
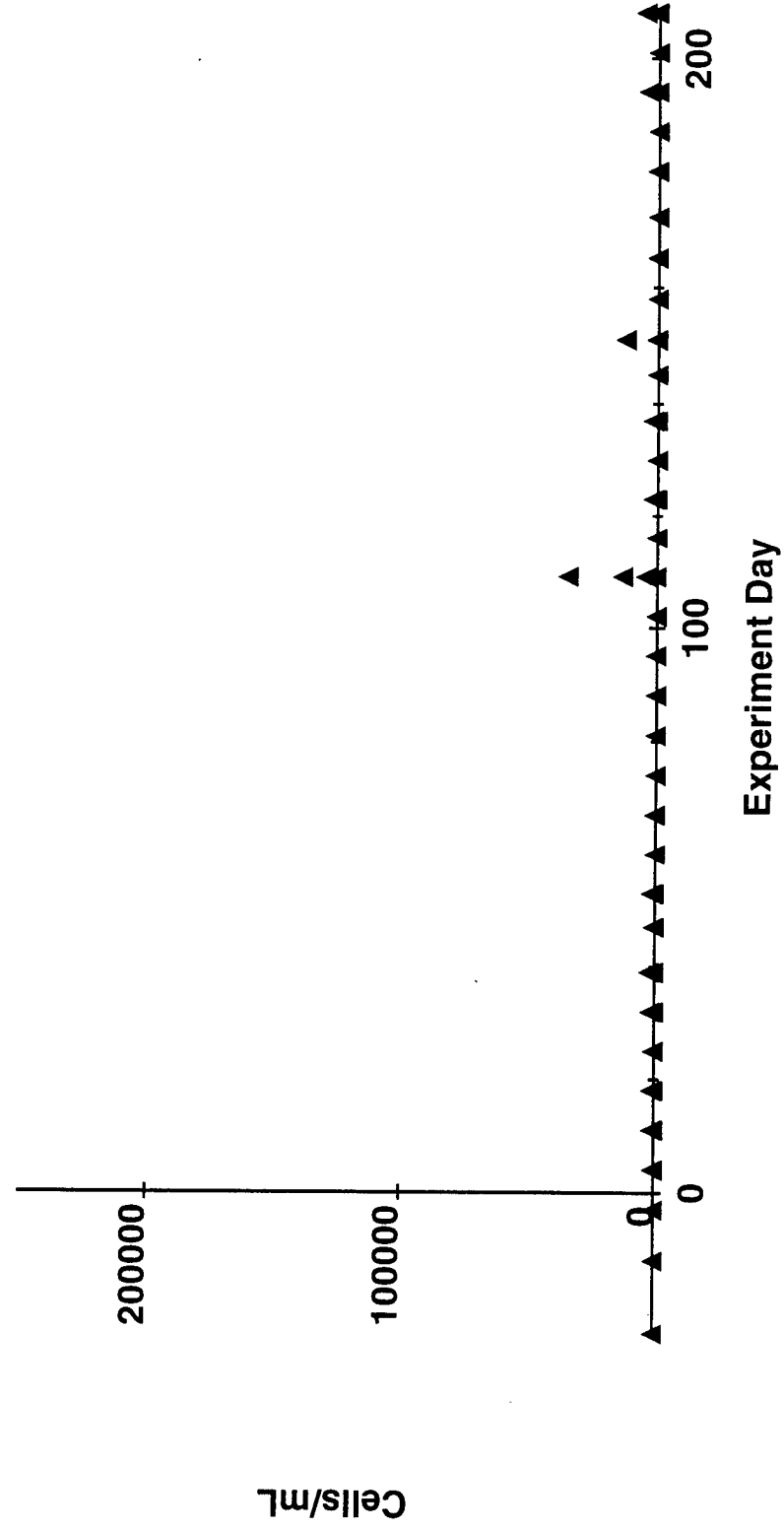
Figure 71d. Phytoplankton - Green Algae
Other - Undosed
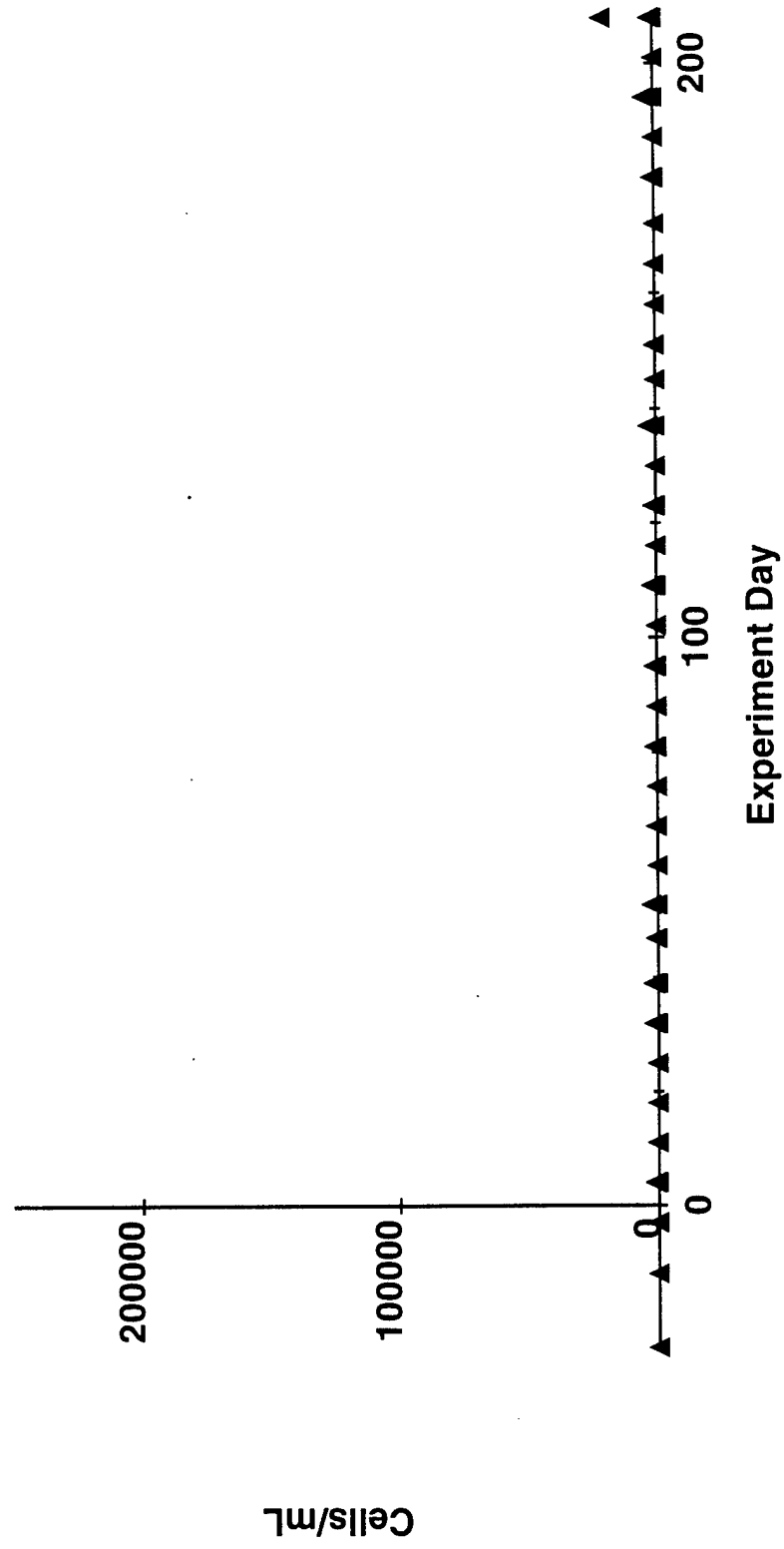
Figure 72a. Phytoplankton - Green Algae
Scenedesmus - High Dose
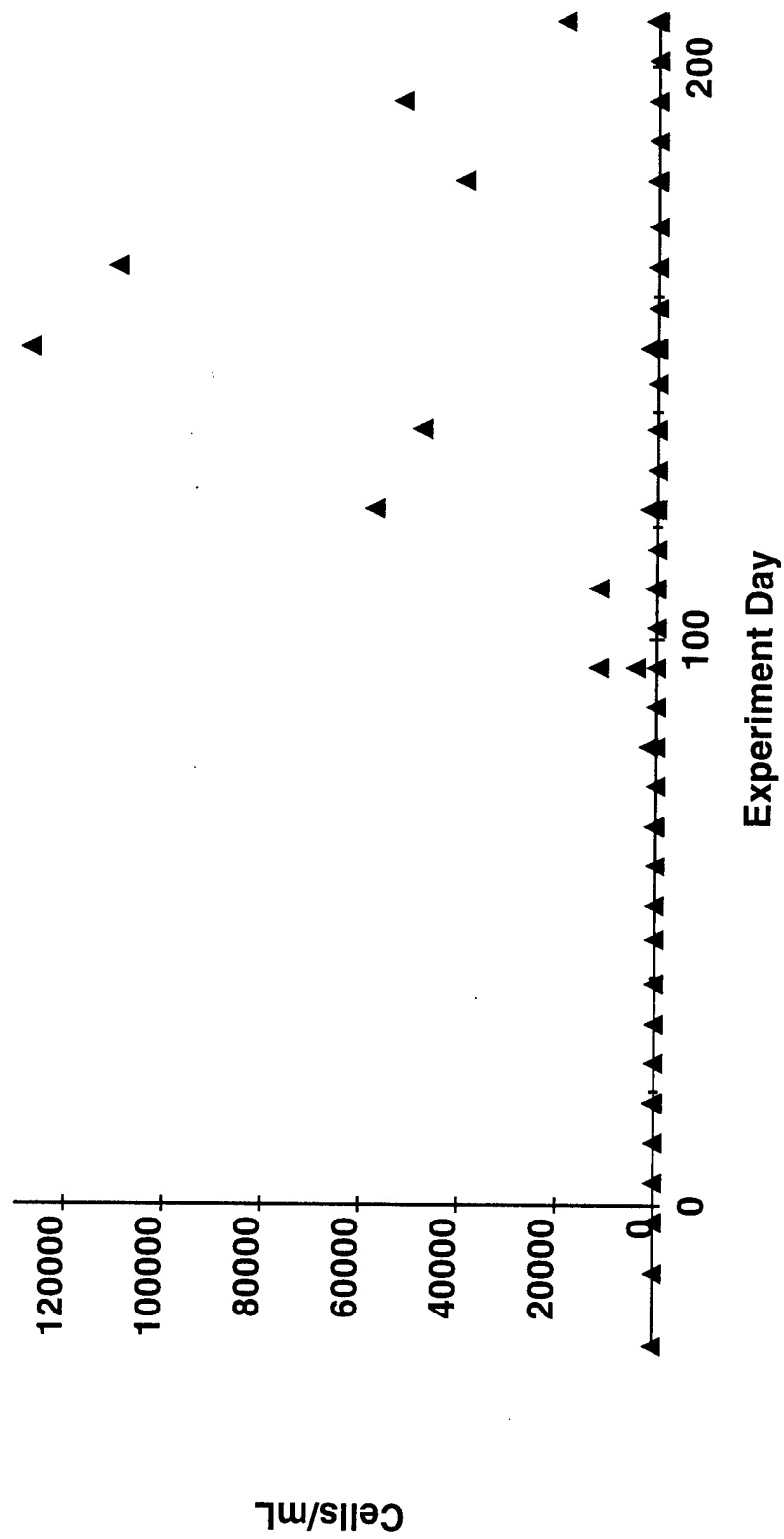
Figure 72b. Phytoplankton - Green Algae
Scenedesmus - Mid-dose
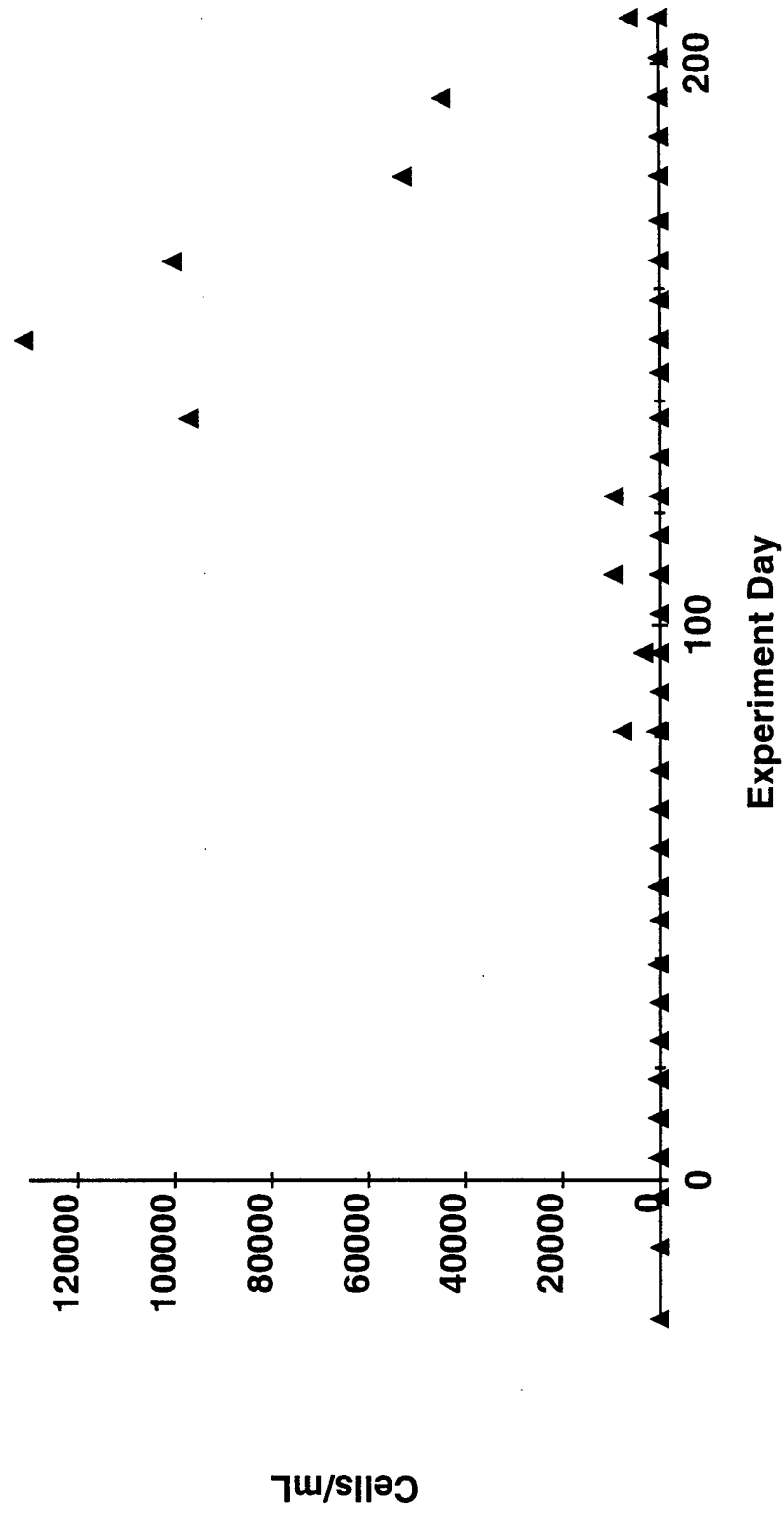
Figure 72c. Phytoplankton - Green Algae
Scenedesmus - Low Dose
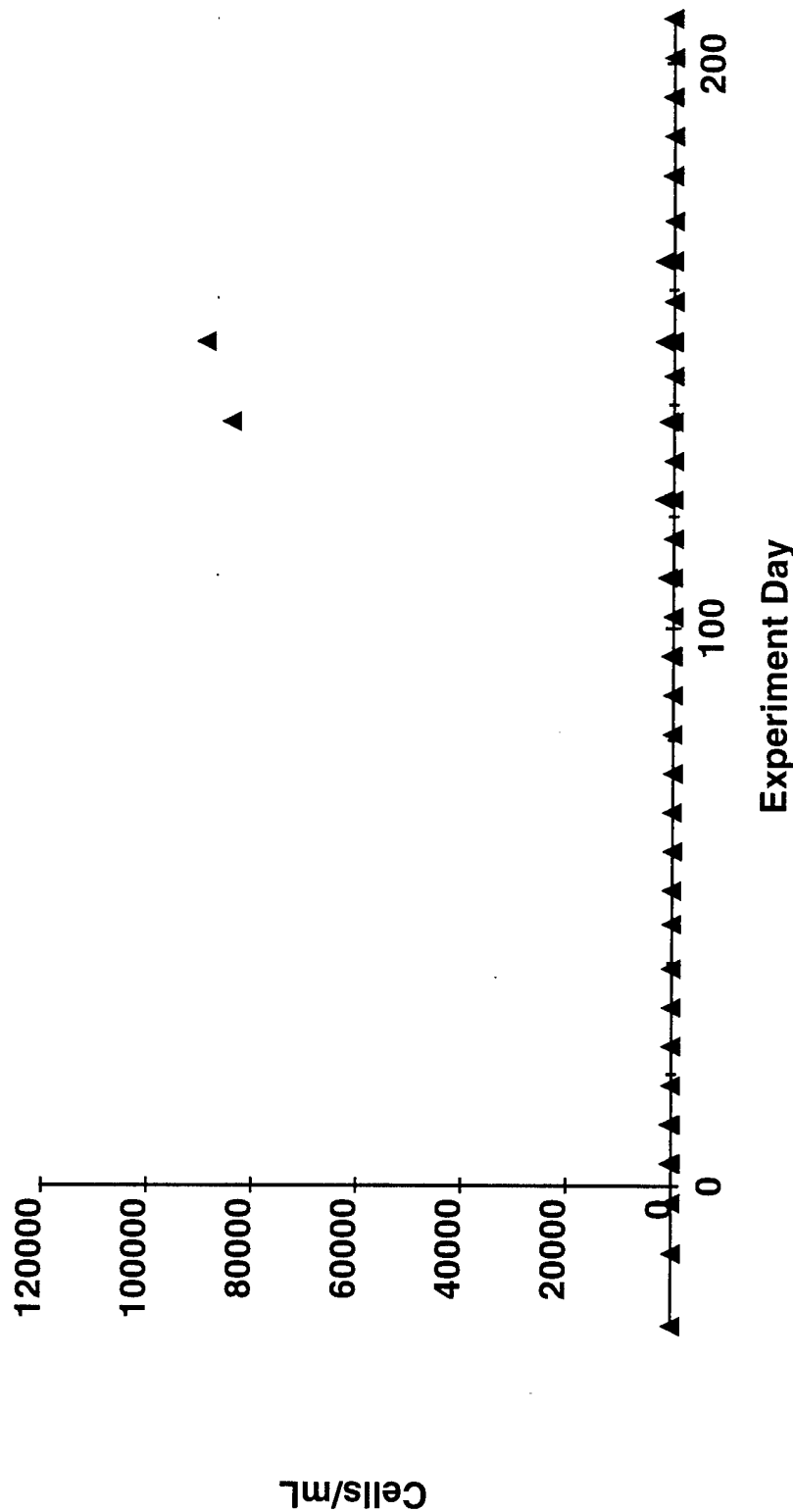
Figure 72d. Phytoplankton - Green Algae
Scenedesmus - Undosed
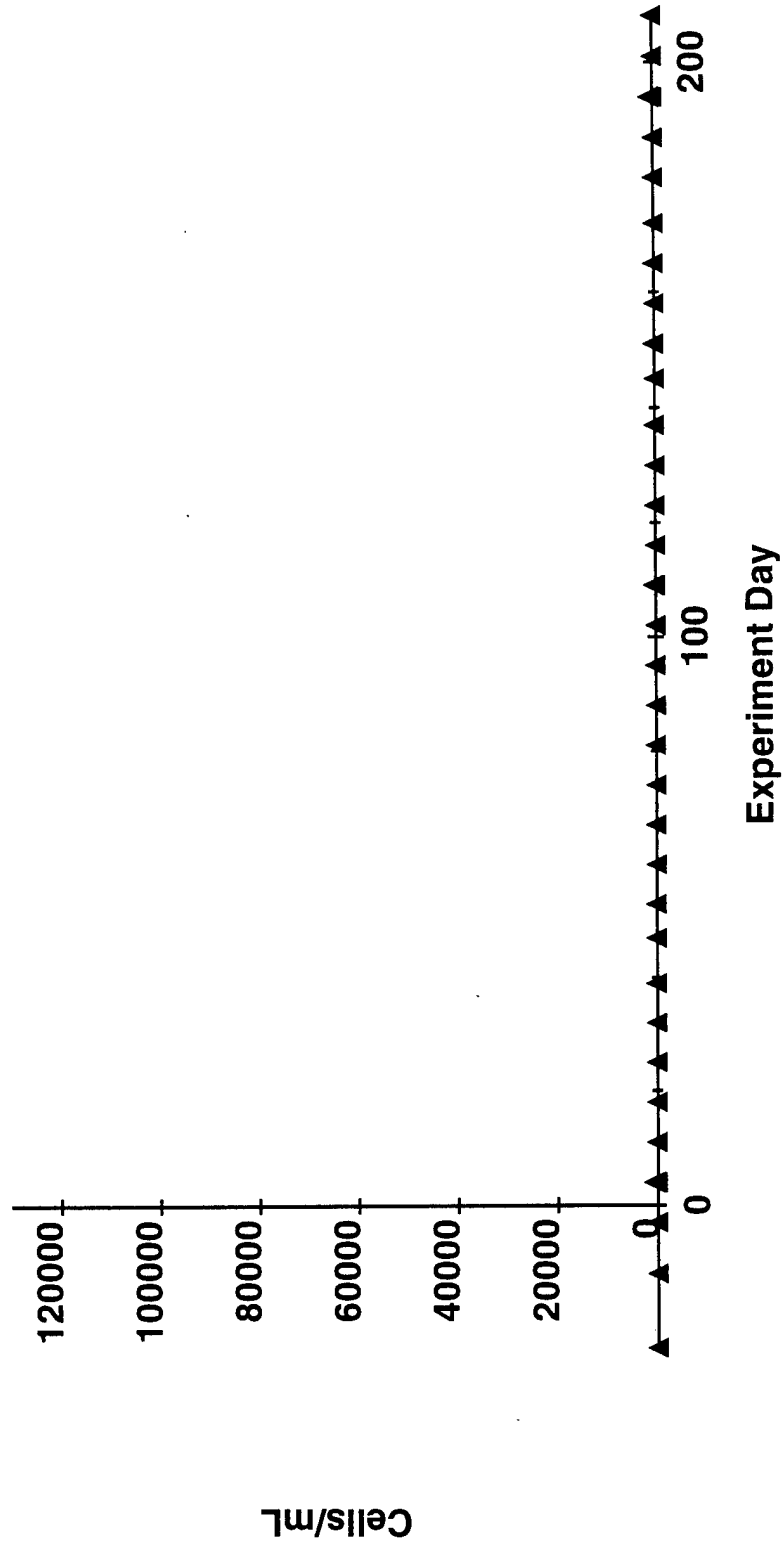
Figure 73a. Phytoplankton - Green Algae
Schroederia - High Dose
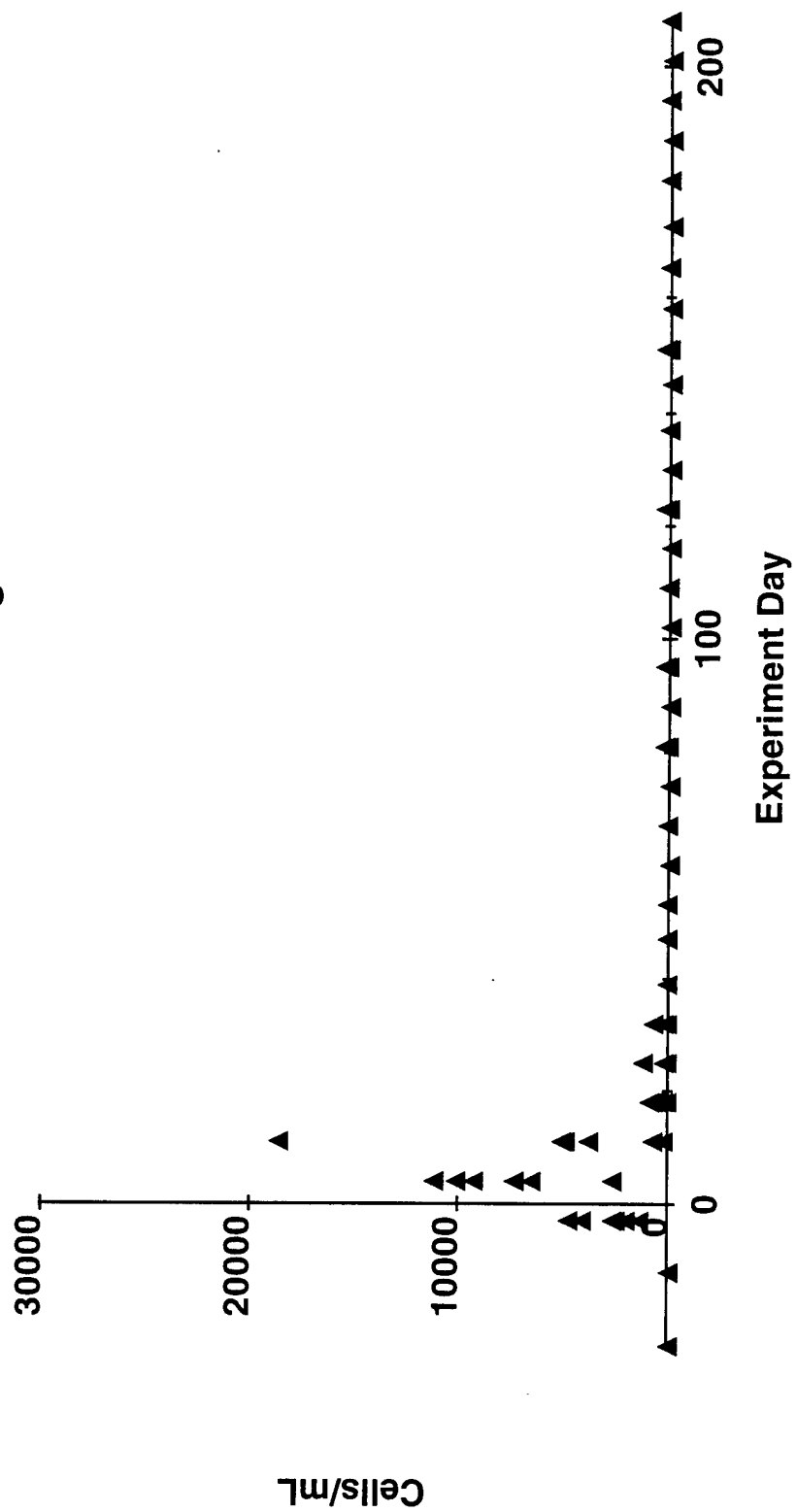
Figure 73b. Phytoplankton - Green Algae
Schroederia - Mid-dose
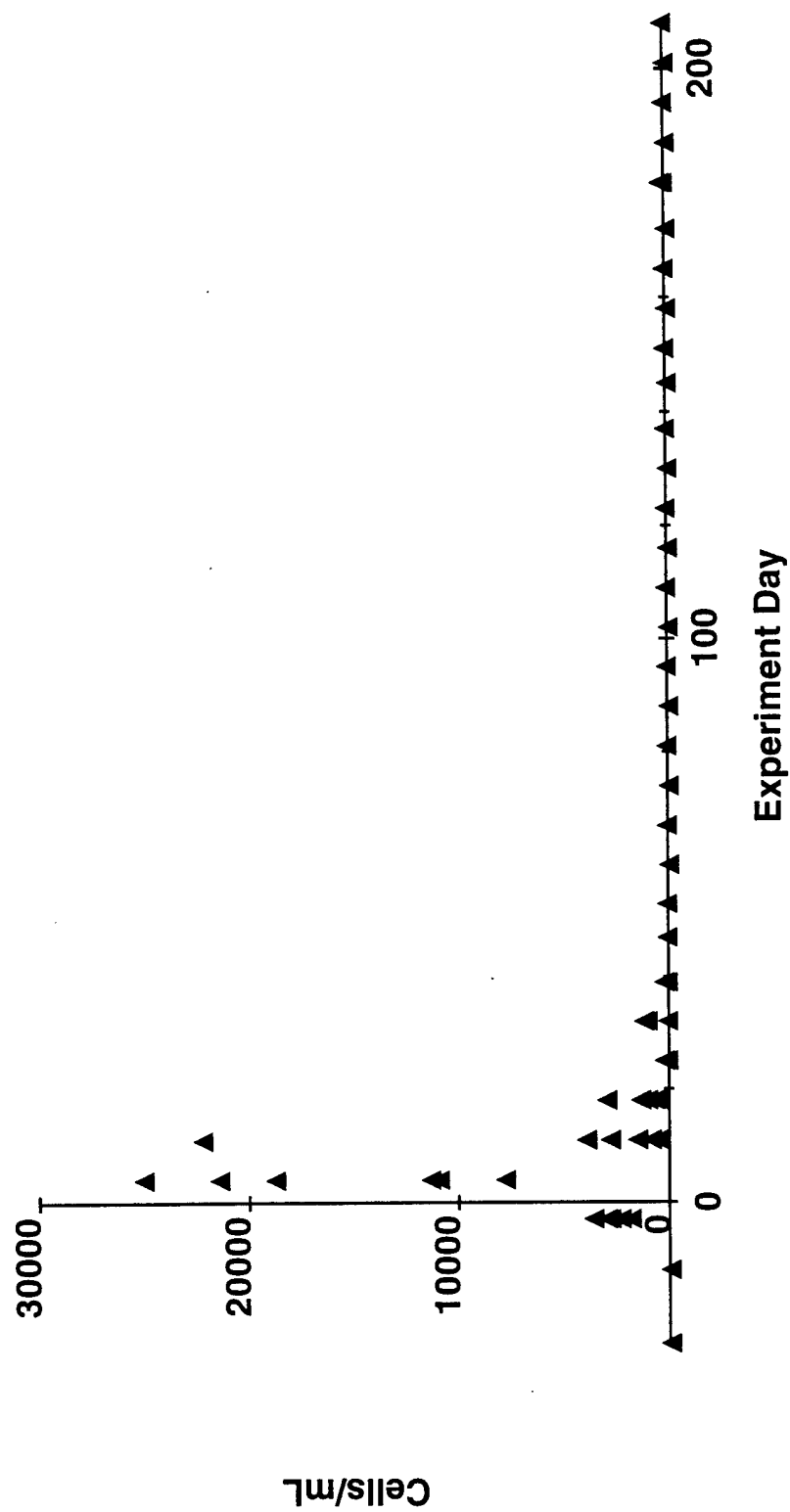
Figure 73c. Phytoplankton - Green Algae
Schroederia - Low Dose
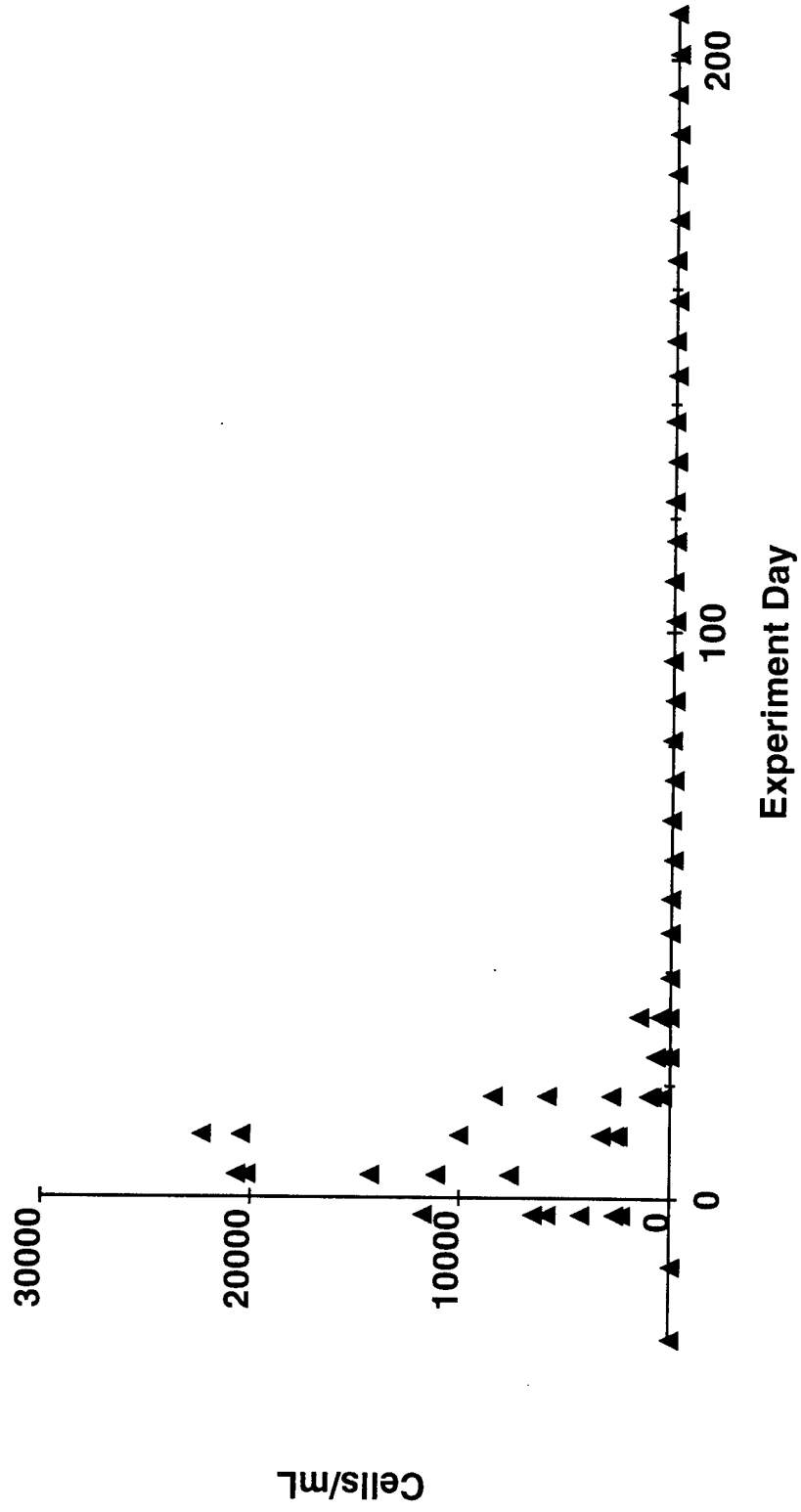
Figure 73d. Phytoplankton - Green Algae
Schroederia - Undosed
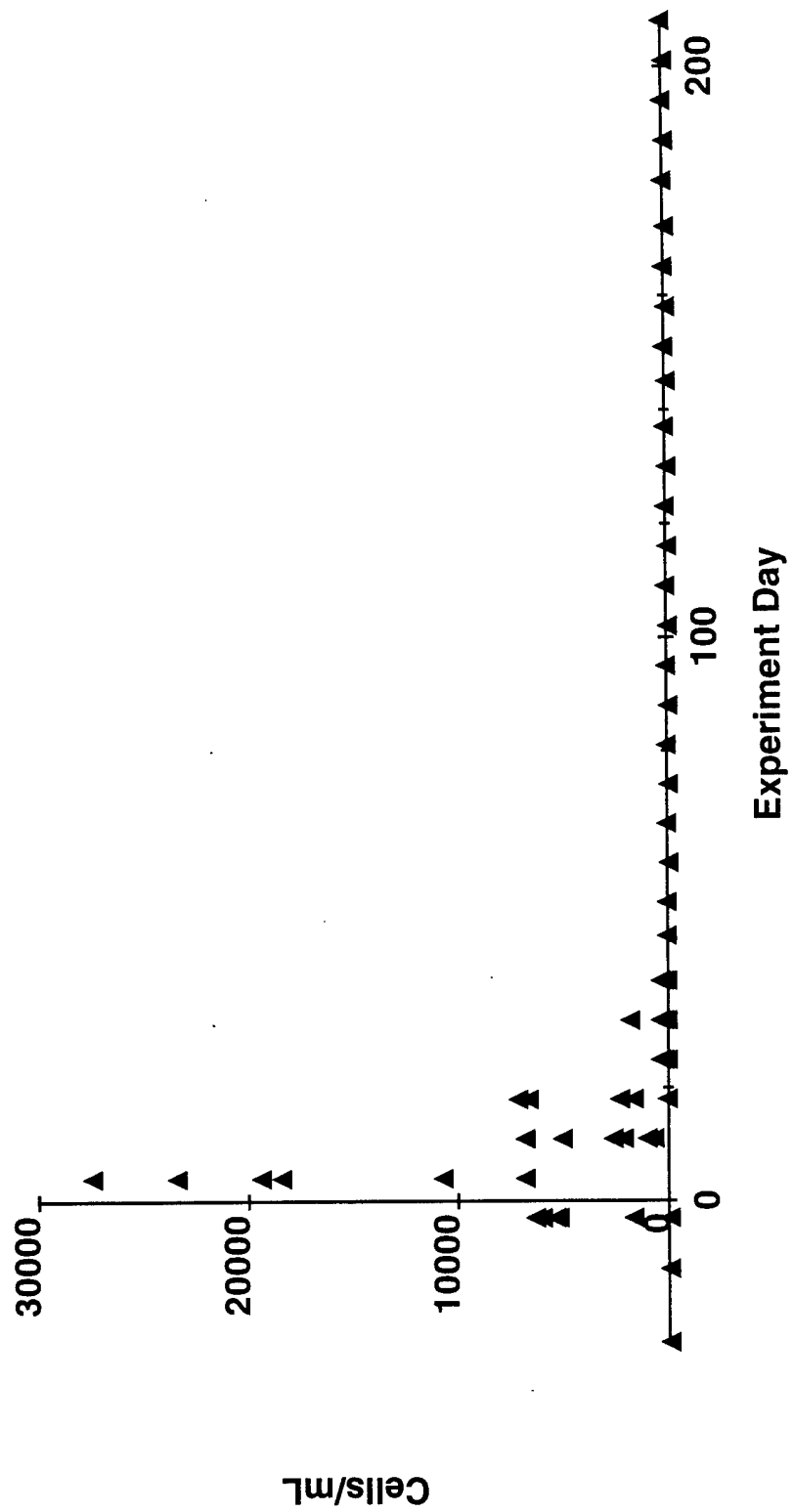
Figure 74a. Phytoplankton - Green Algae
Selenastrum - High Dose
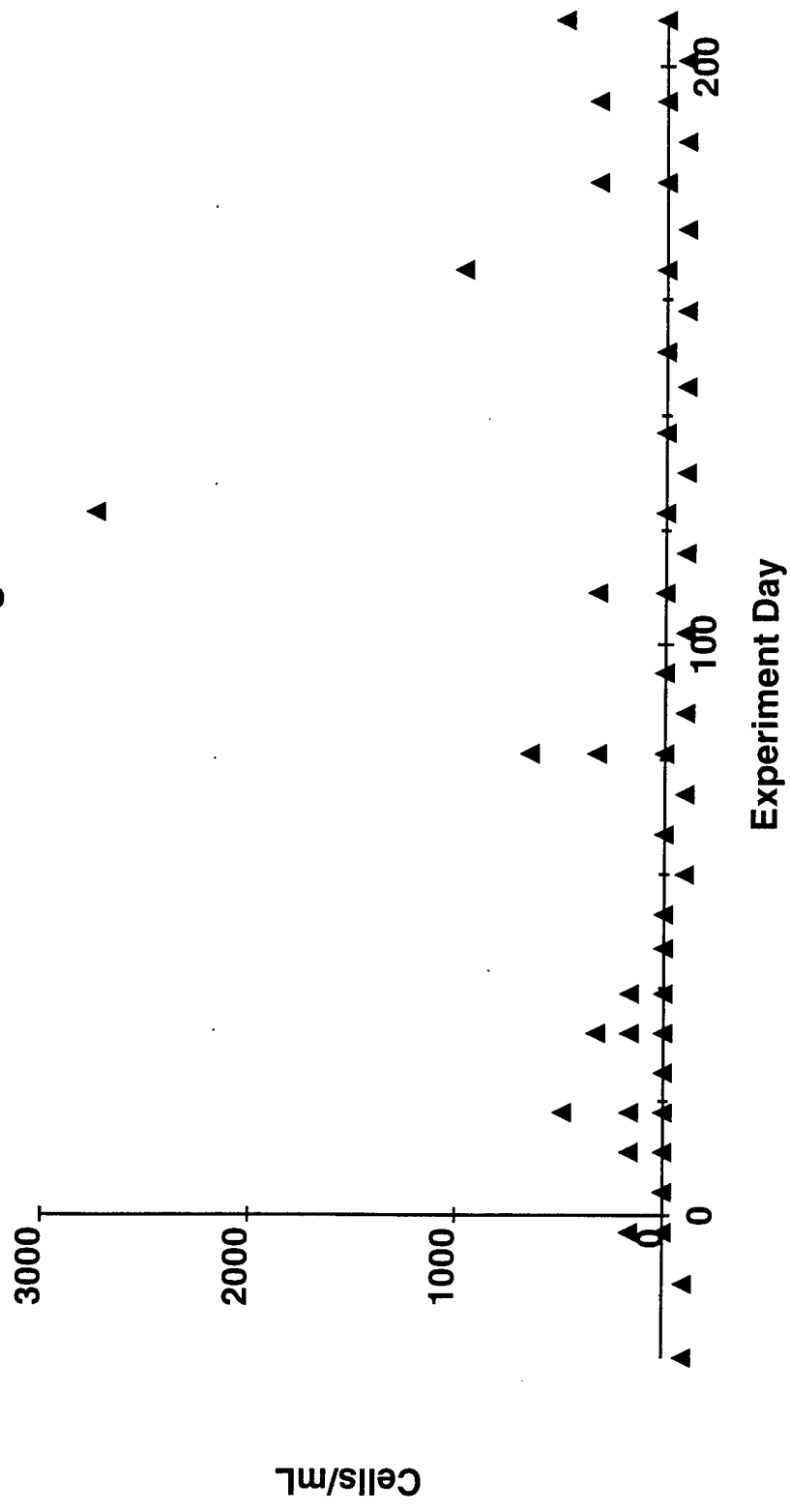
Figure 74b. Phytoplankton - Green Algae
Selenastrum - Mid-dose
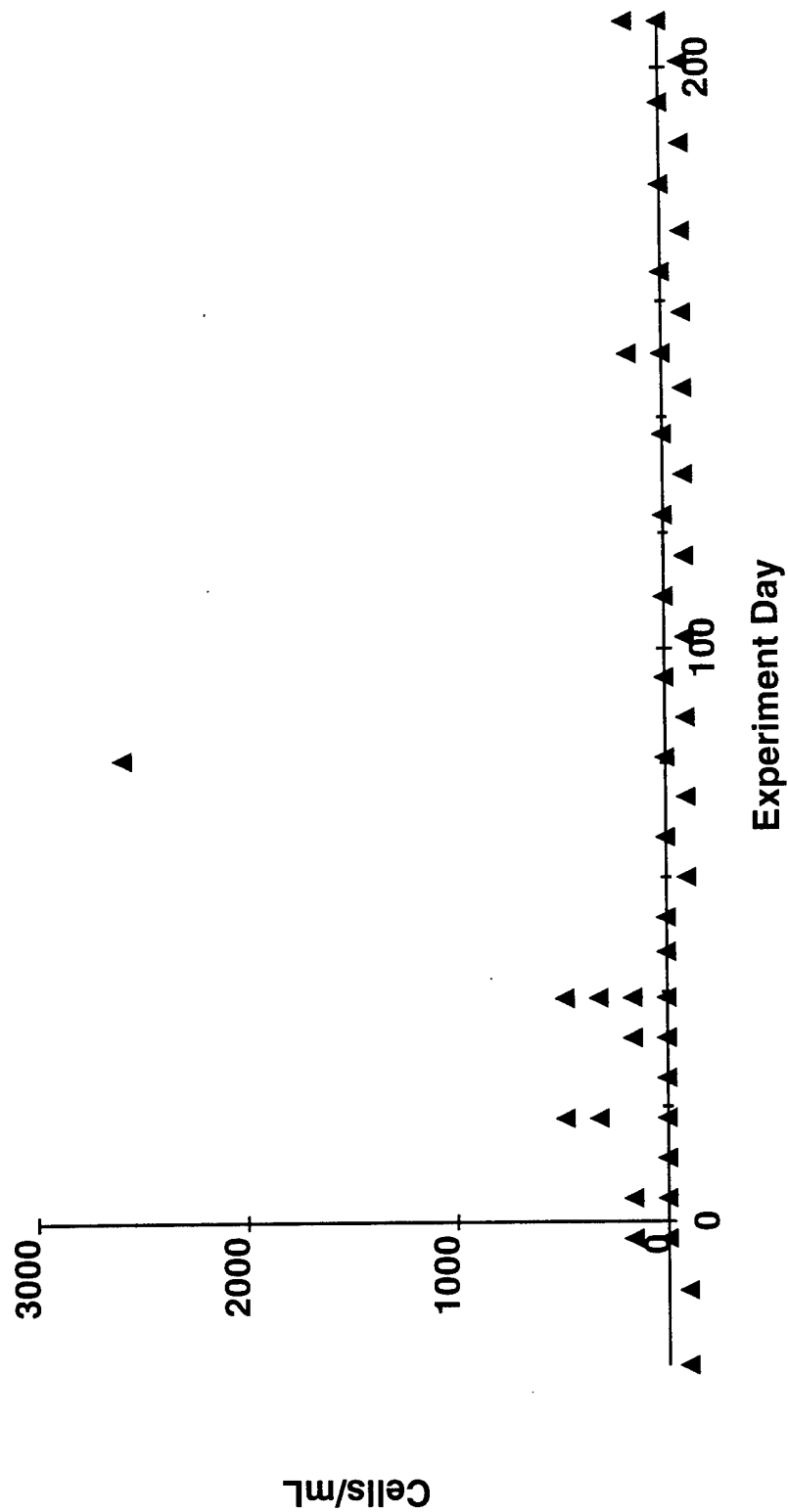
Figure 74c. Phytoplankton - Green Algae
Selenastrum - Low Dose
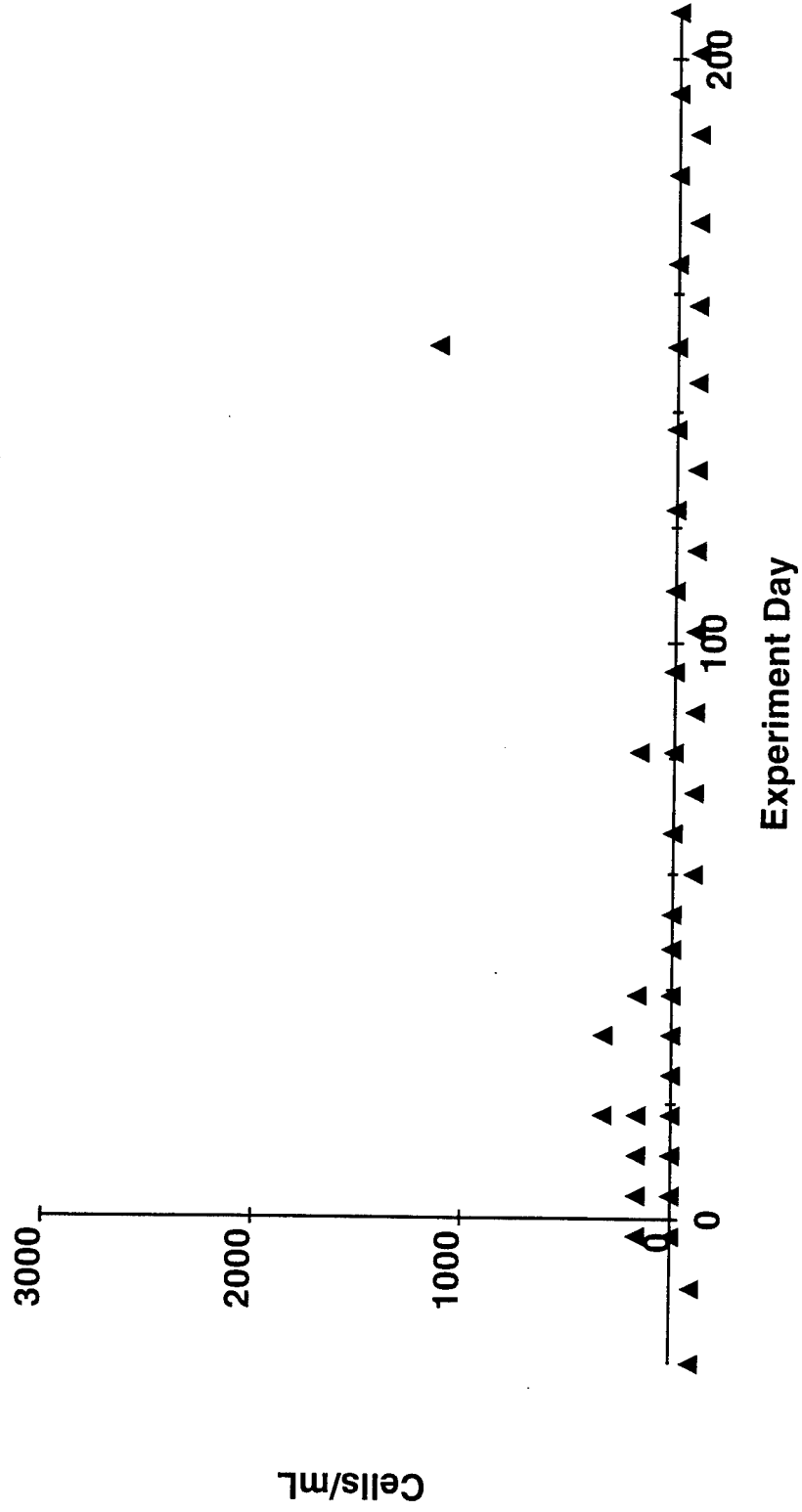
Figure 74d. Phytoplankton - Green Algae
Selenastrum - Undosed

Cells/mL

Experiment Day
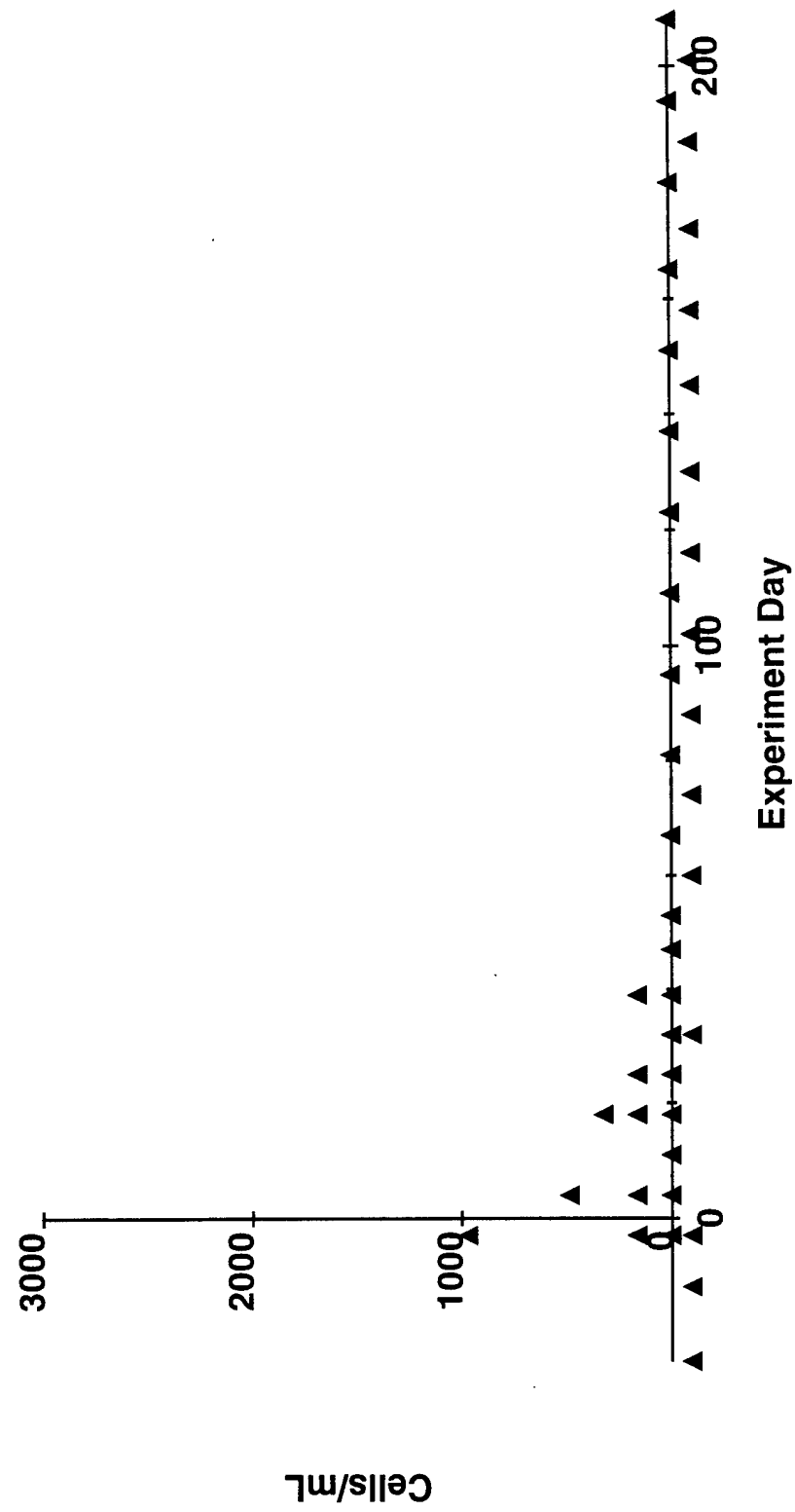
Figure 75a. Phytoplankton - Miscellaneous
Amoebas - High Dose
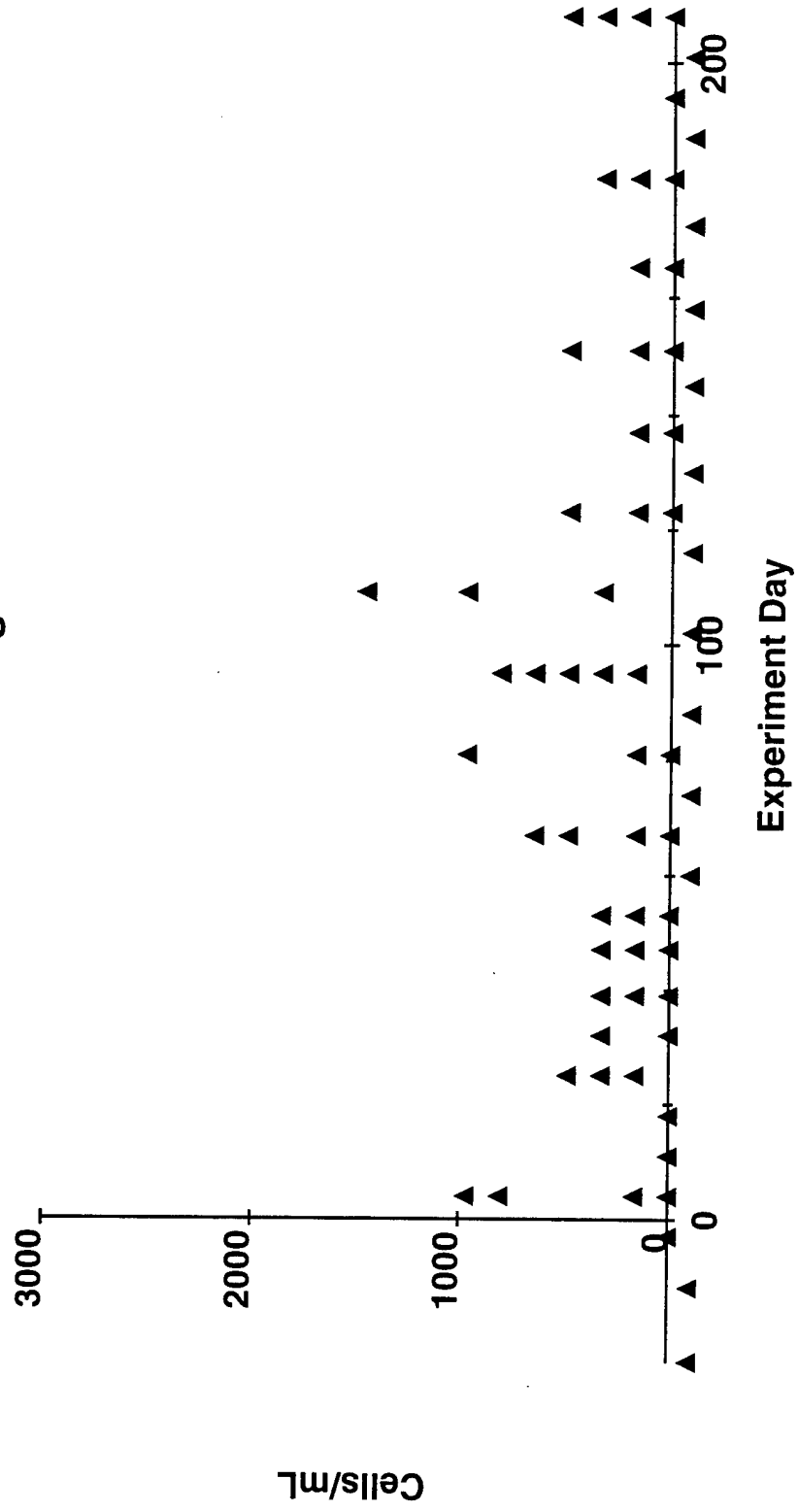
Figure 75b. Phytoplankton - Miscellaneous
Amoebas - Mid-dose
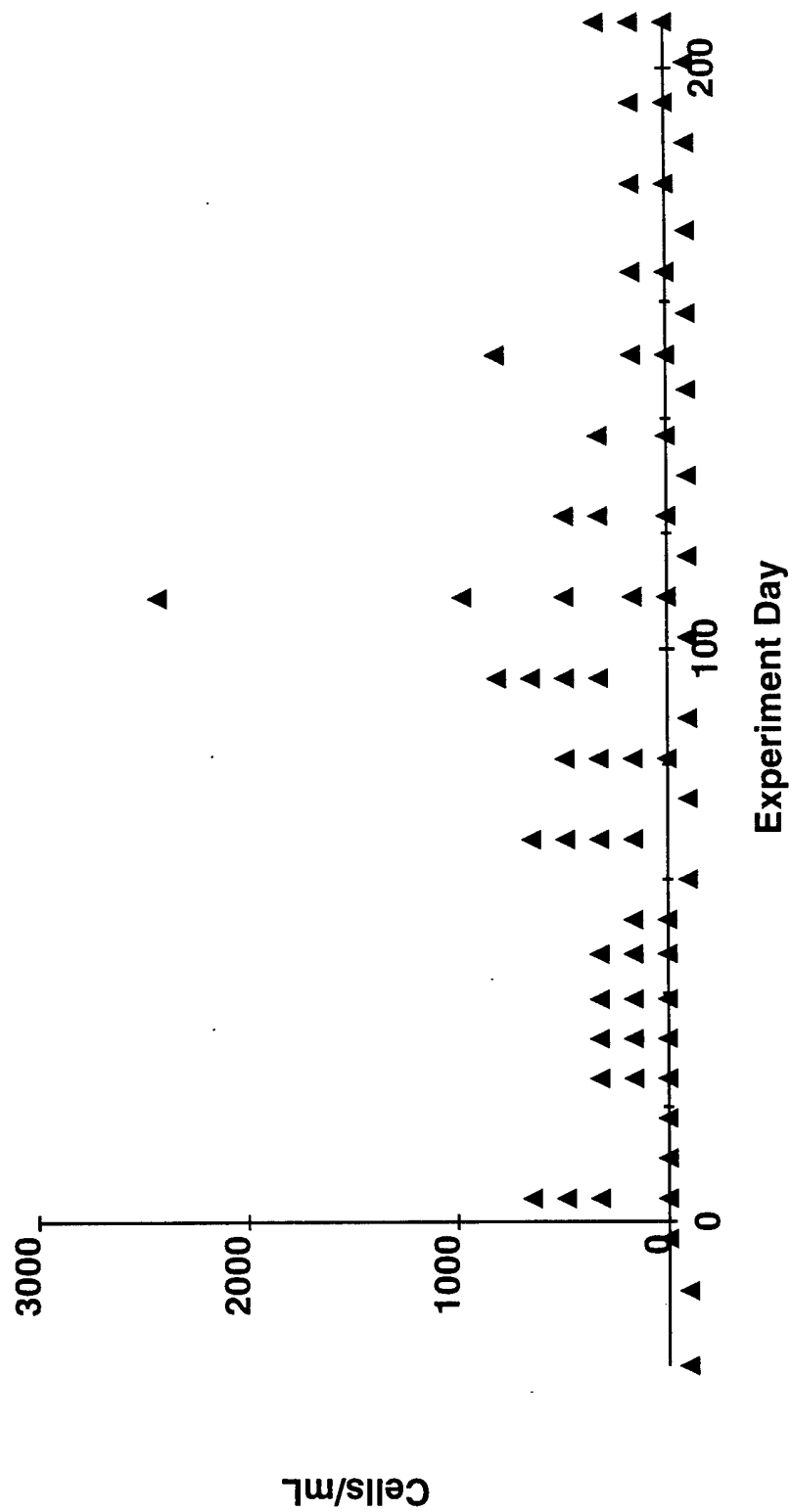
Figure 75c. Phytoplankton - Miscellaneous
Amoebas - Low Dose
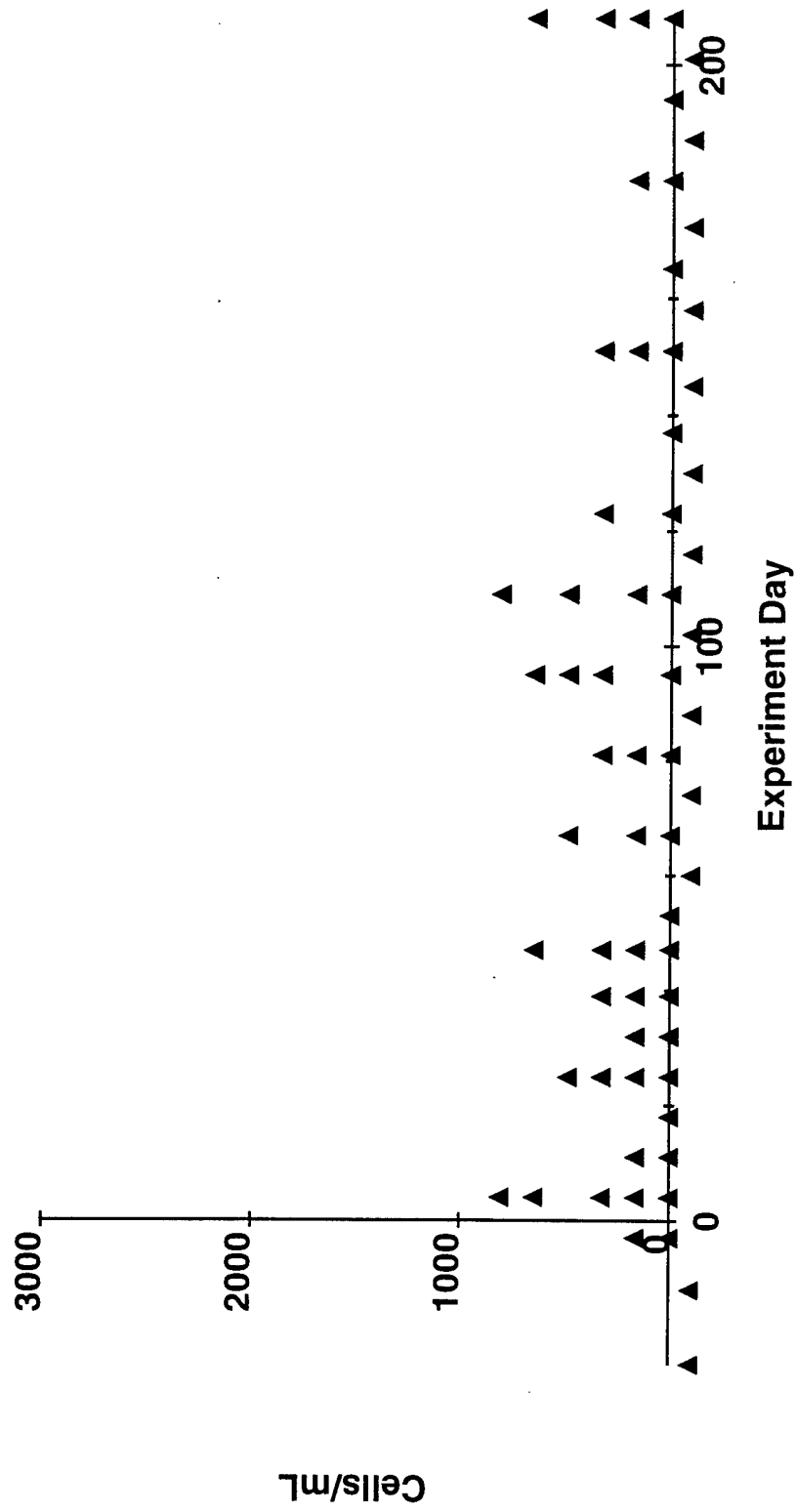
Figure 75d. Phytoplankton - Miscellaneous
Amoebas - Undosed
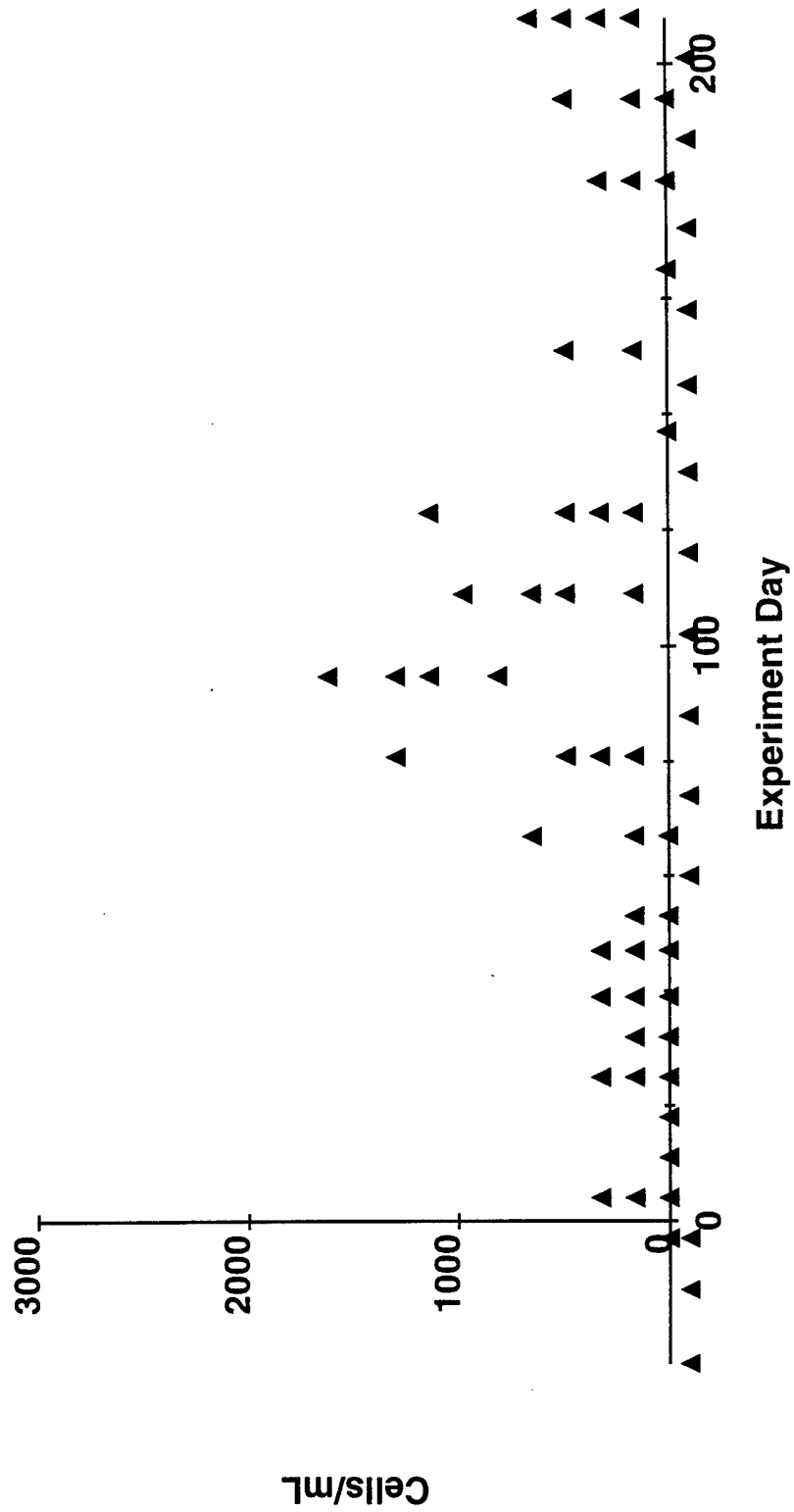
Figure 76a. Phytoplankton - Miscellaneous
Rotifers - High Dose
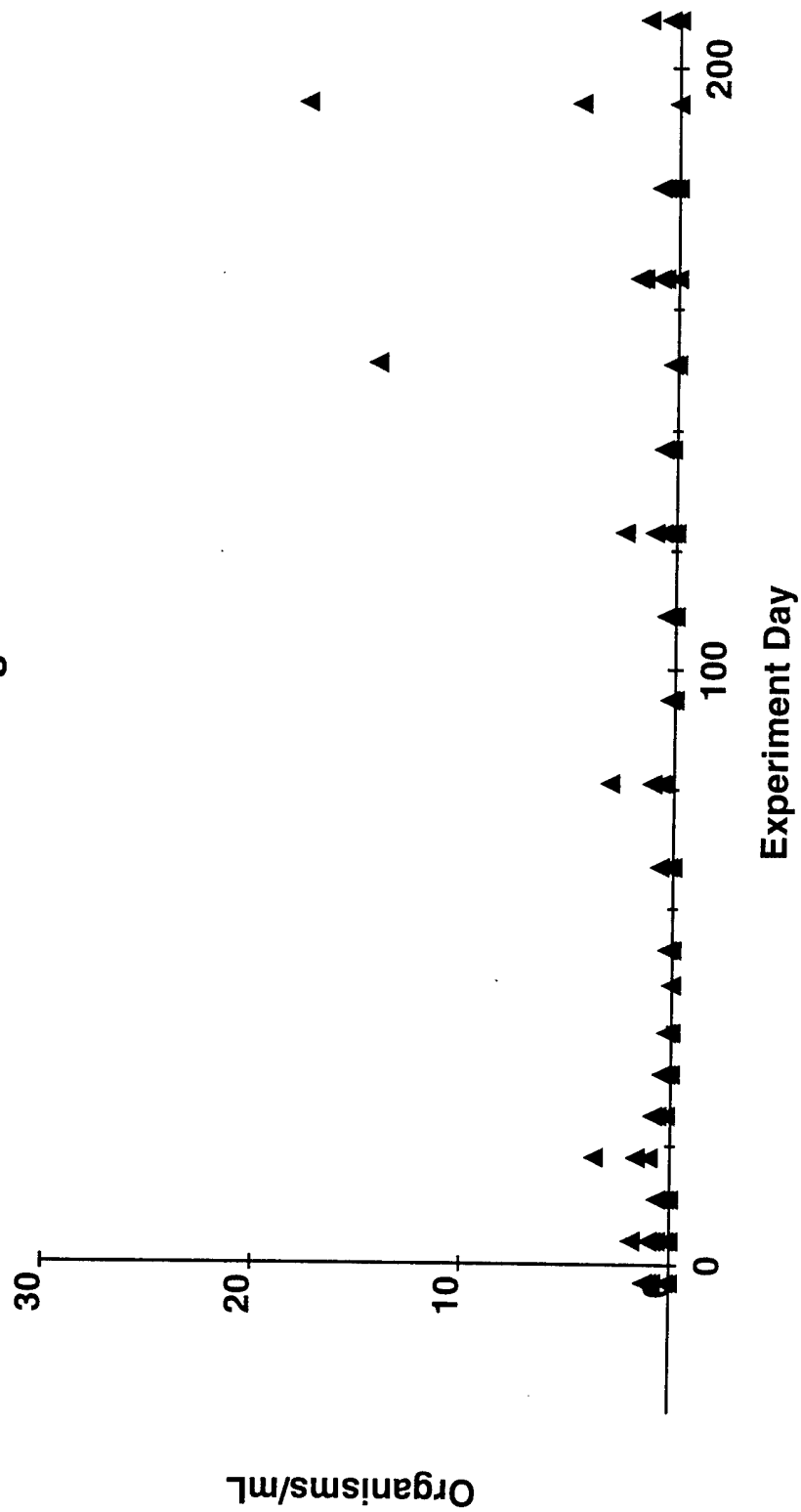
Figure 76b. Phytoplankton - Miscellaneous
Rotifers - Mid-dose
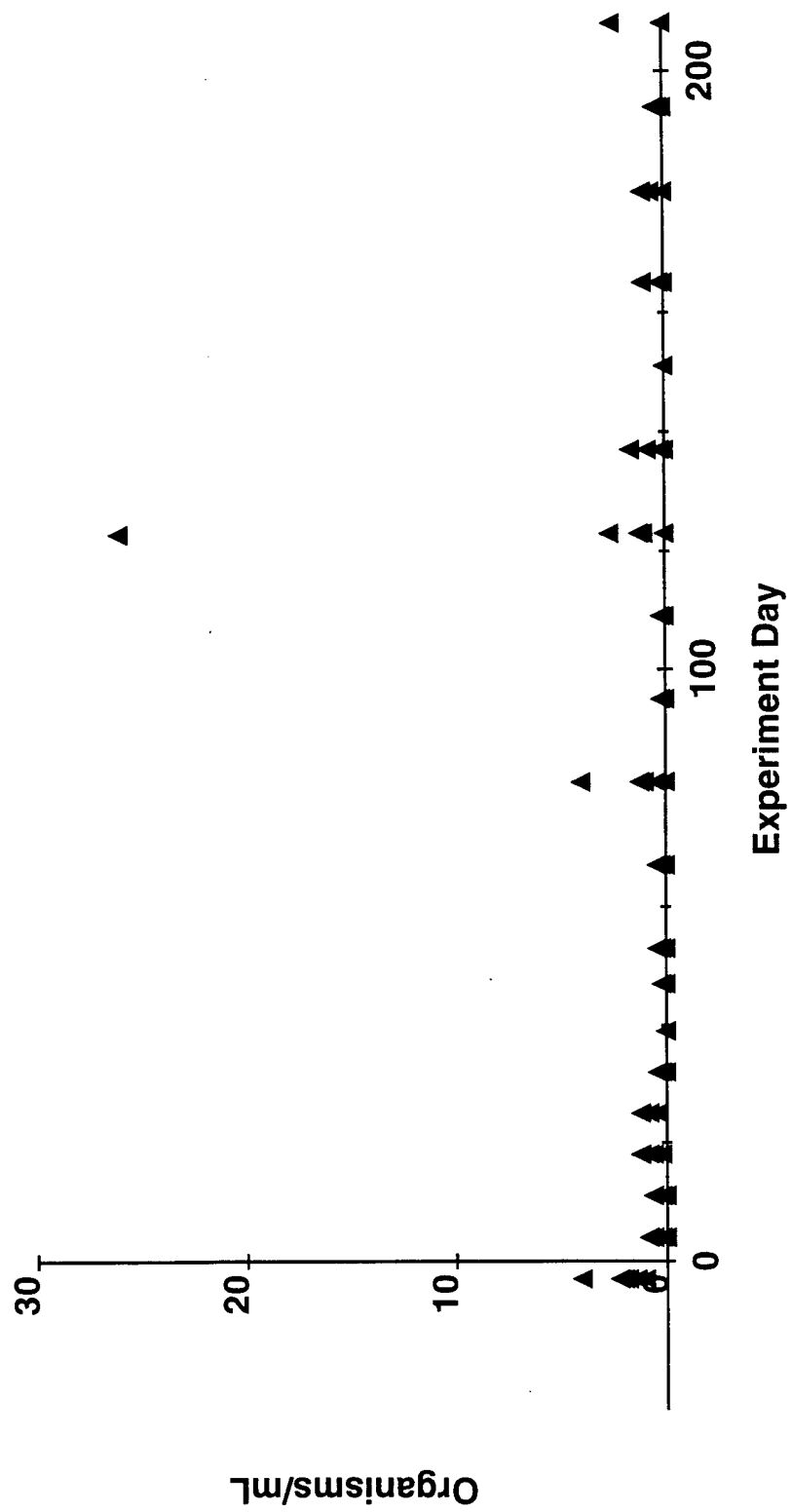
Figure 76c. Phytoplankton - Miscellaneous
Rotifers - Low Dose
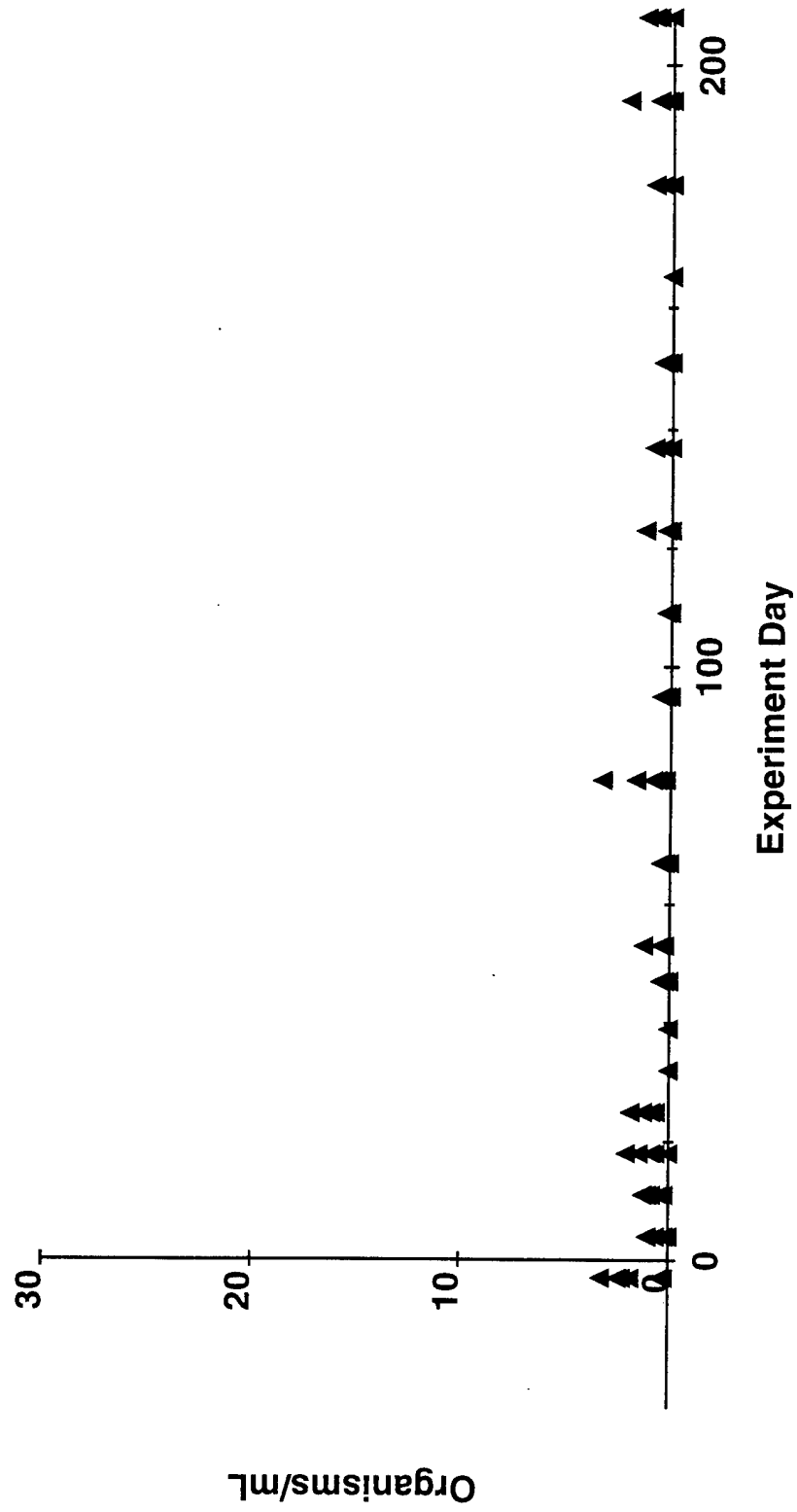
Figure 76d. Phytoplankton - Miscellaneous
Rotifers - Undosed
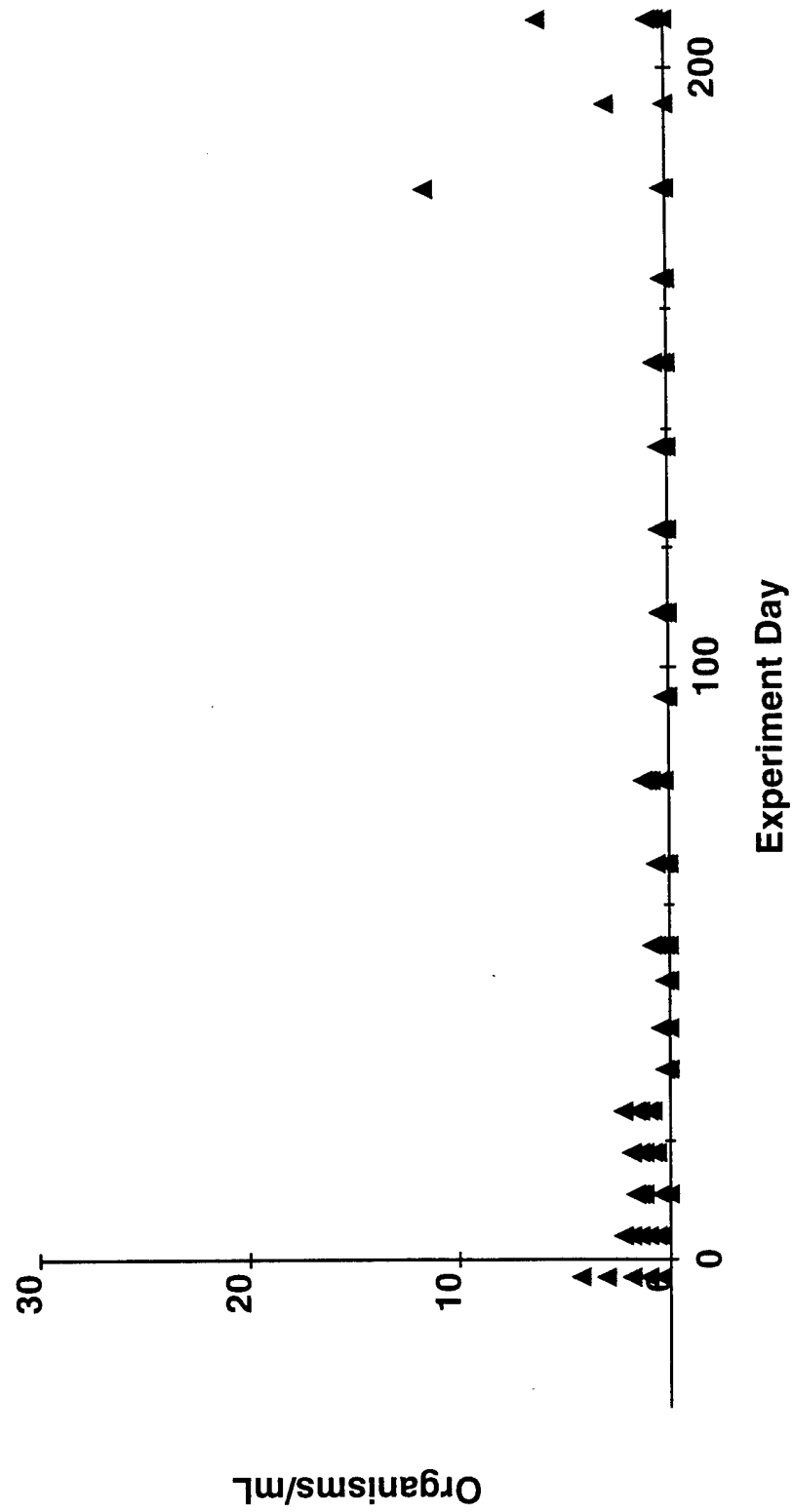
Figure 77a. Phytoplankton
Ash-Free Dry Weight - High Dose
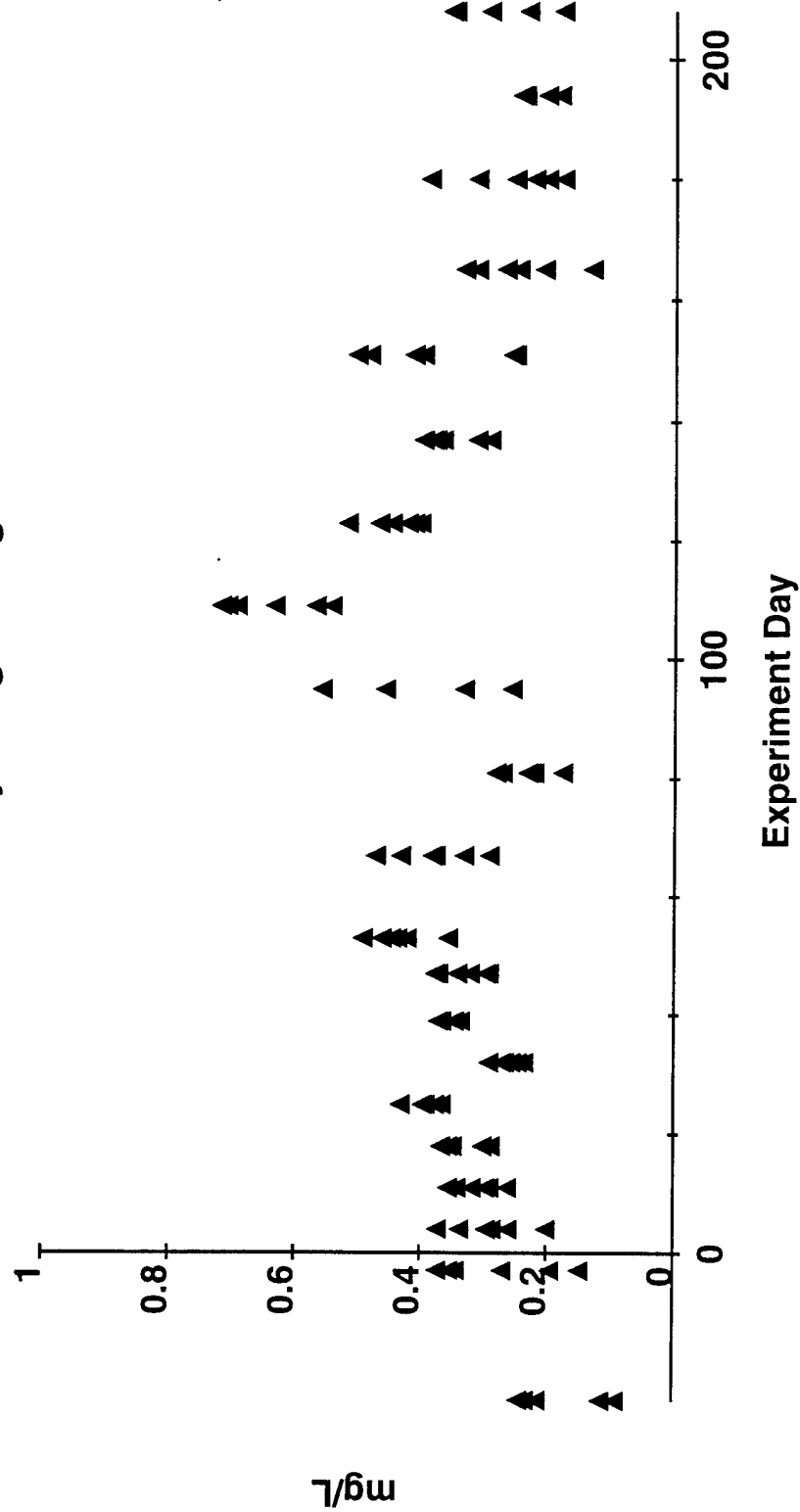
Figure 77b. Phytoplankton
Ash-Free Dry Weight - Mid-dose
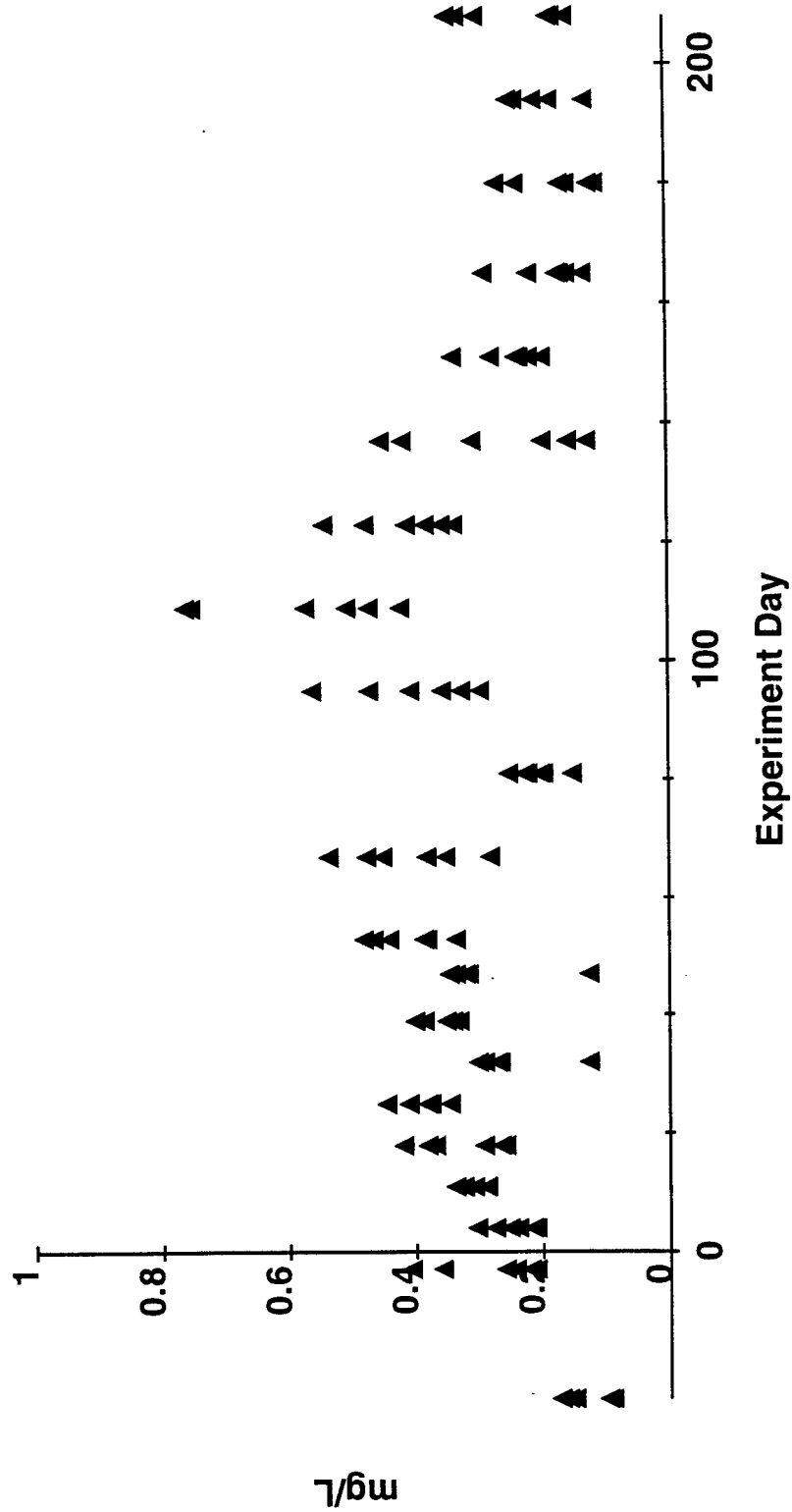
Figure 77c. Phytoplankton
Ash-Free Dry Weight - Low Dose
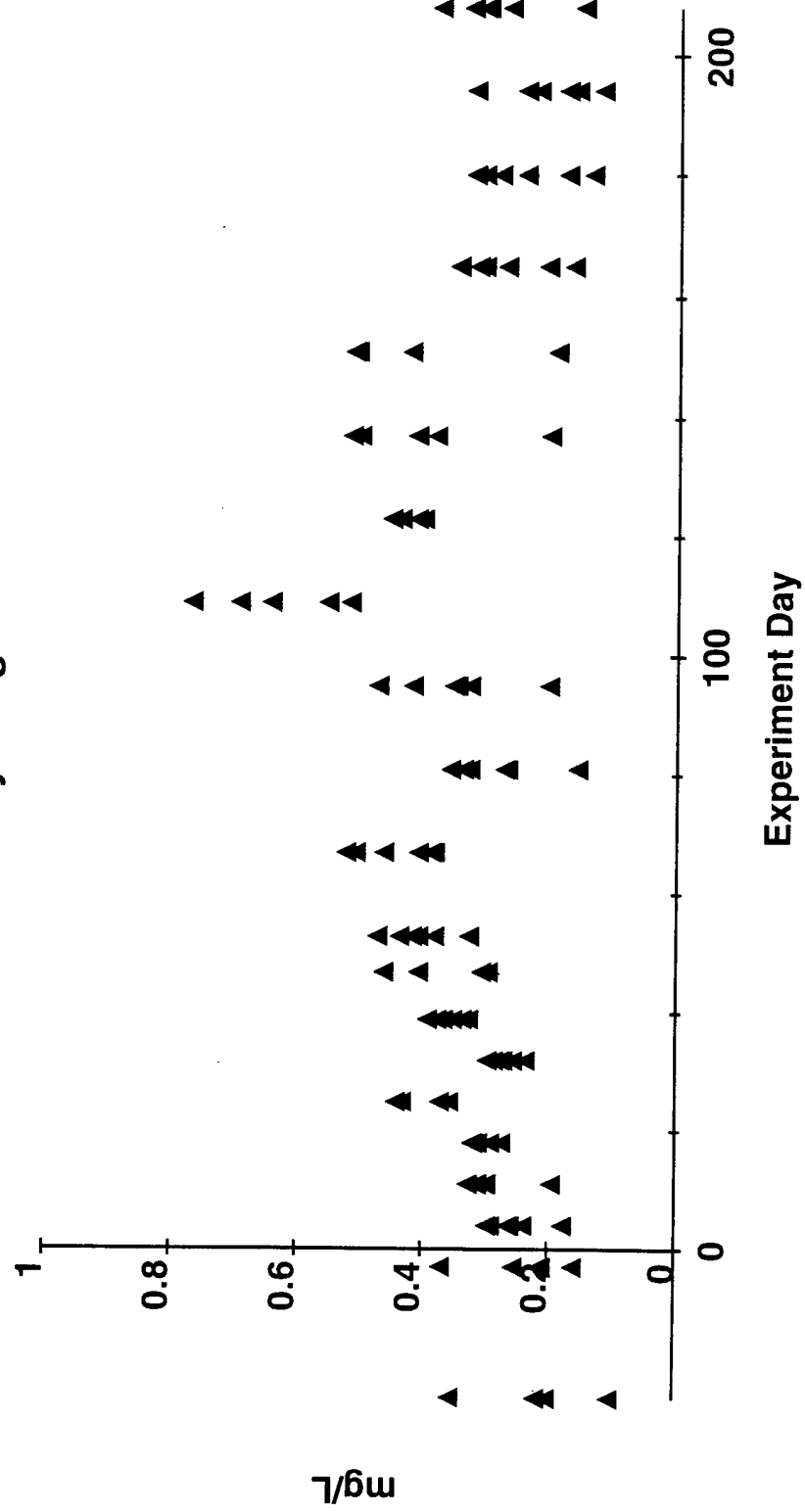
Figure 77d. Phytoplankton
Ash-Free Dry Weight - Undosed
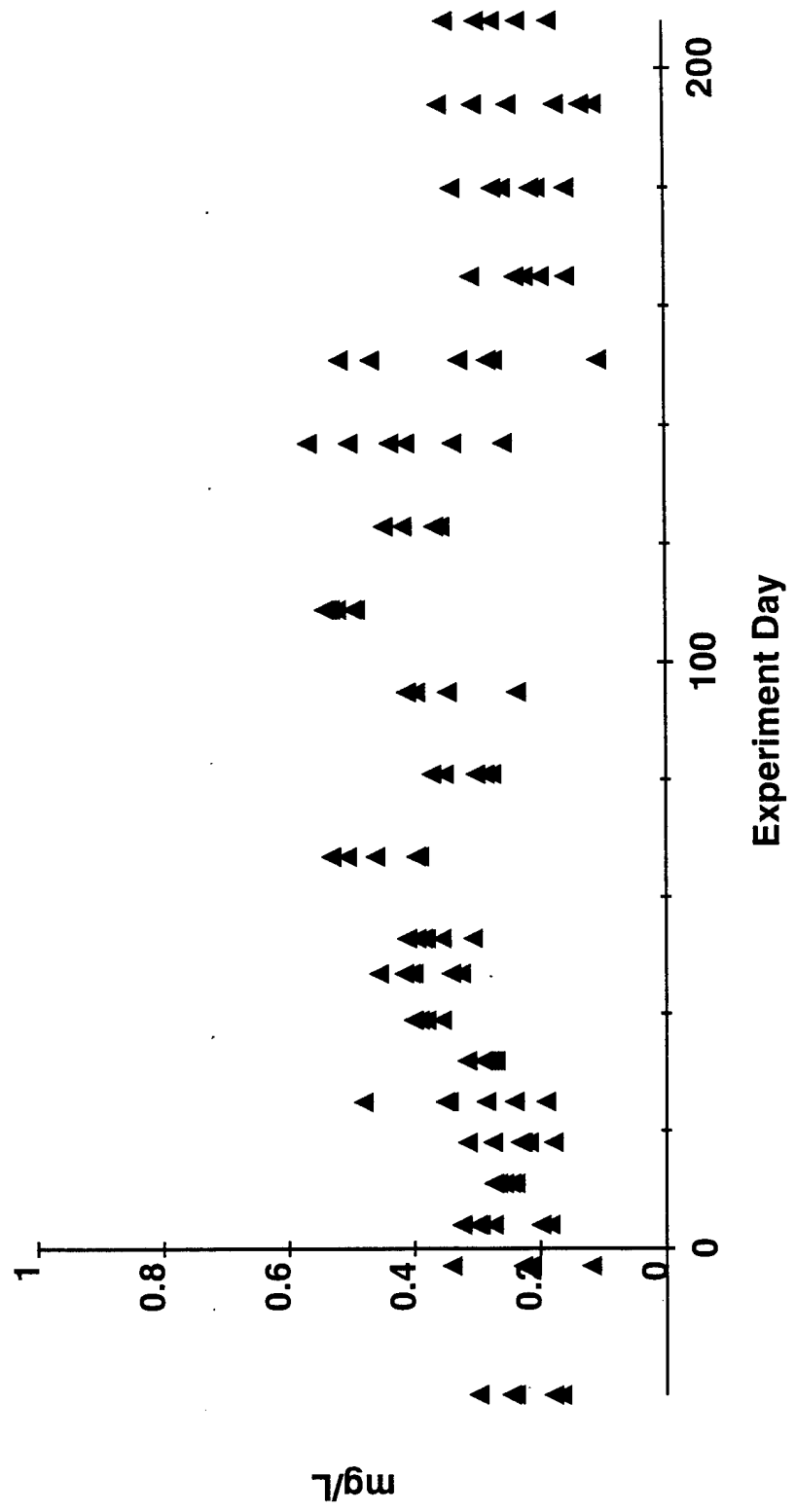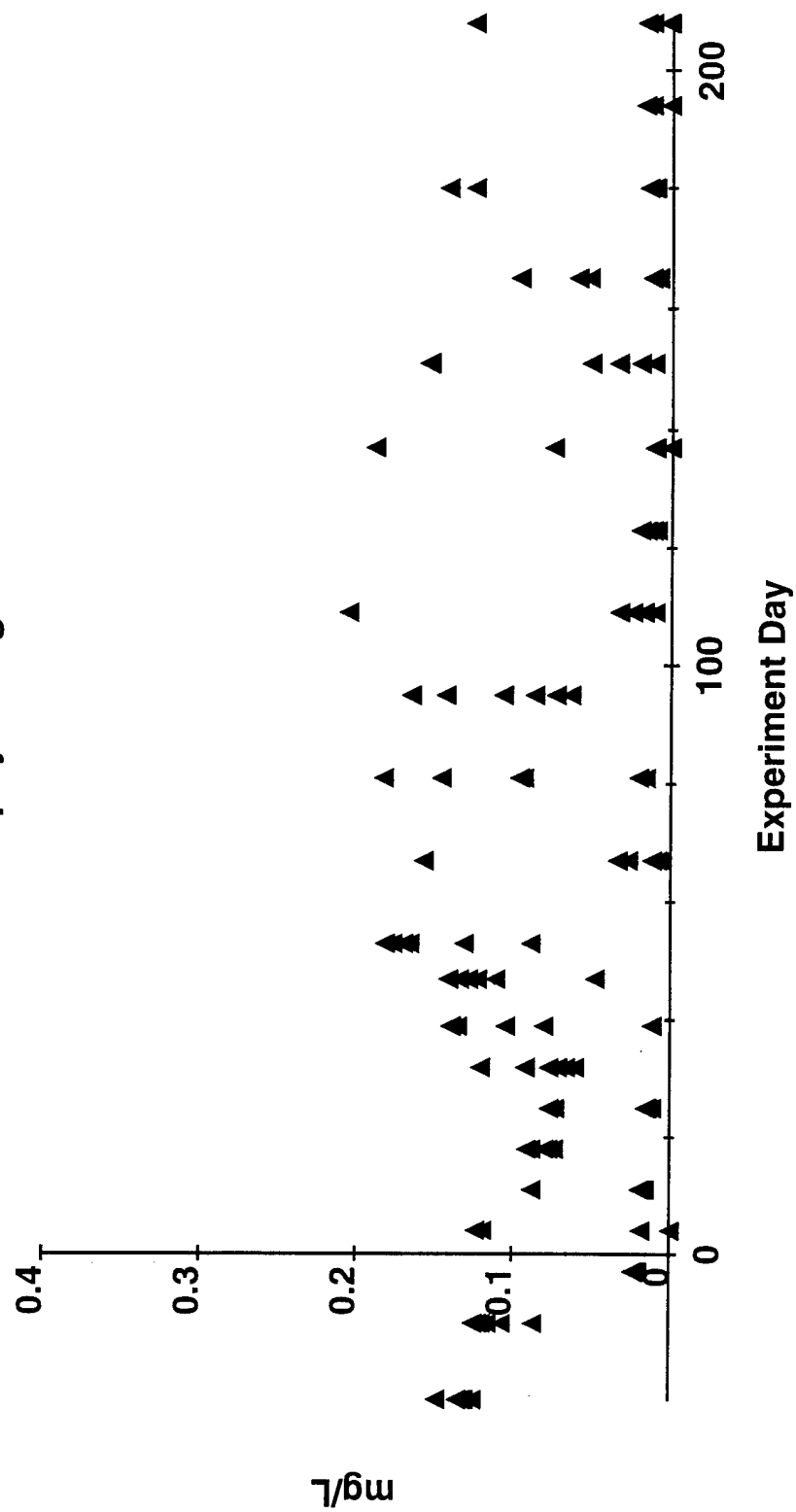
Figure 78a. Phytoplankton
Chlorophyll - High Dose

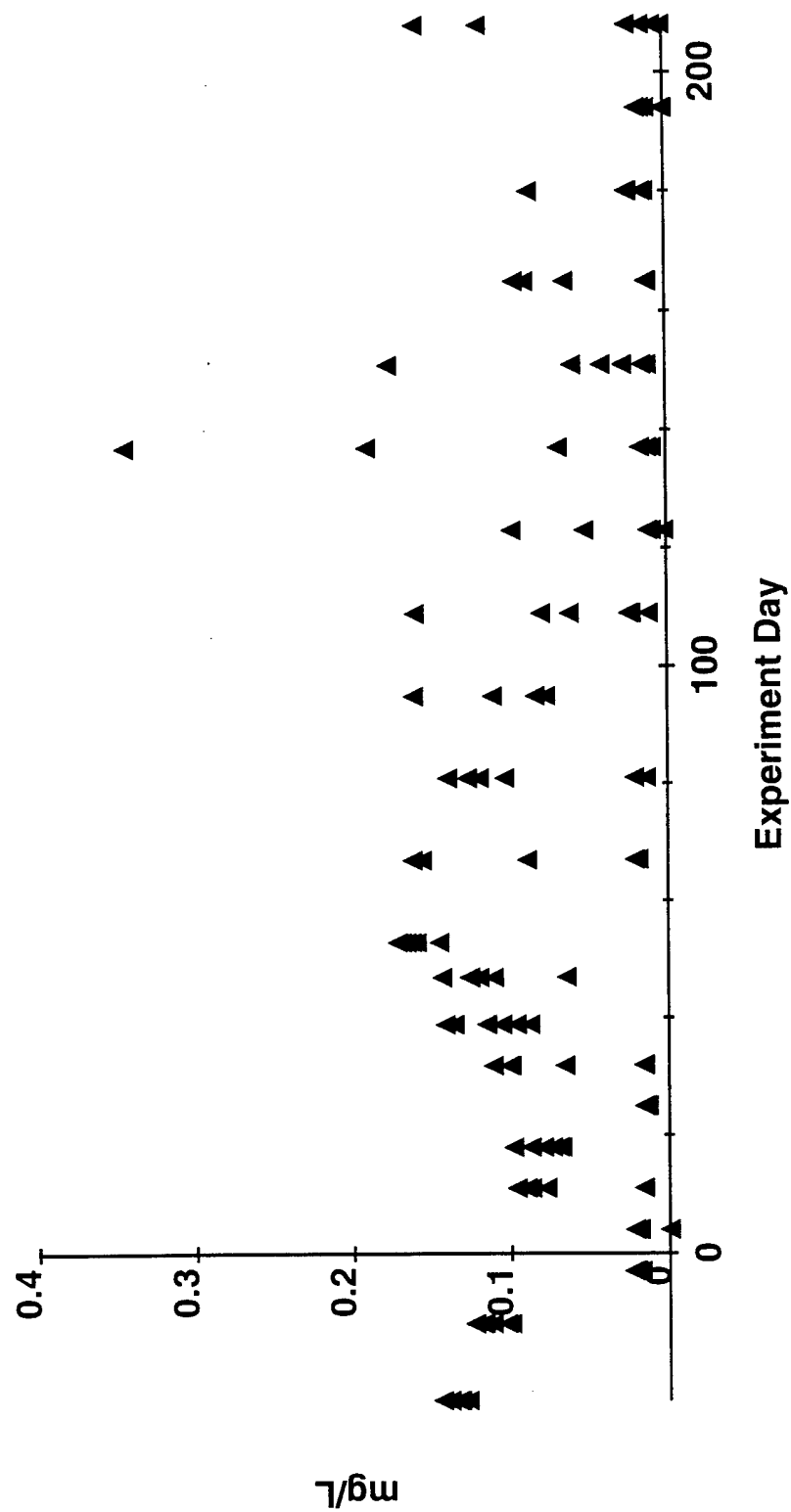Figure 78b. Phytoplankton
Chlorophyll - Mid-dose
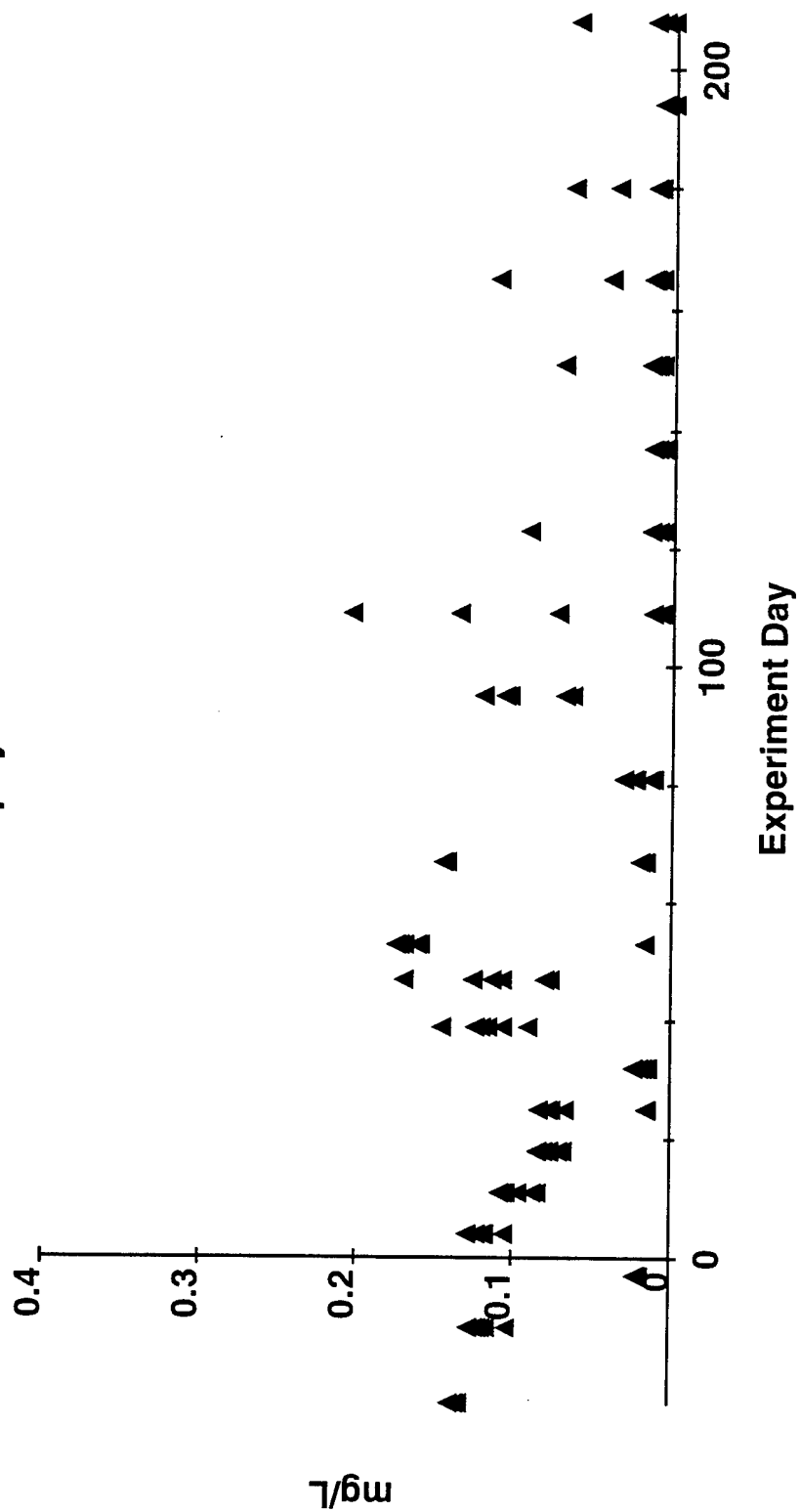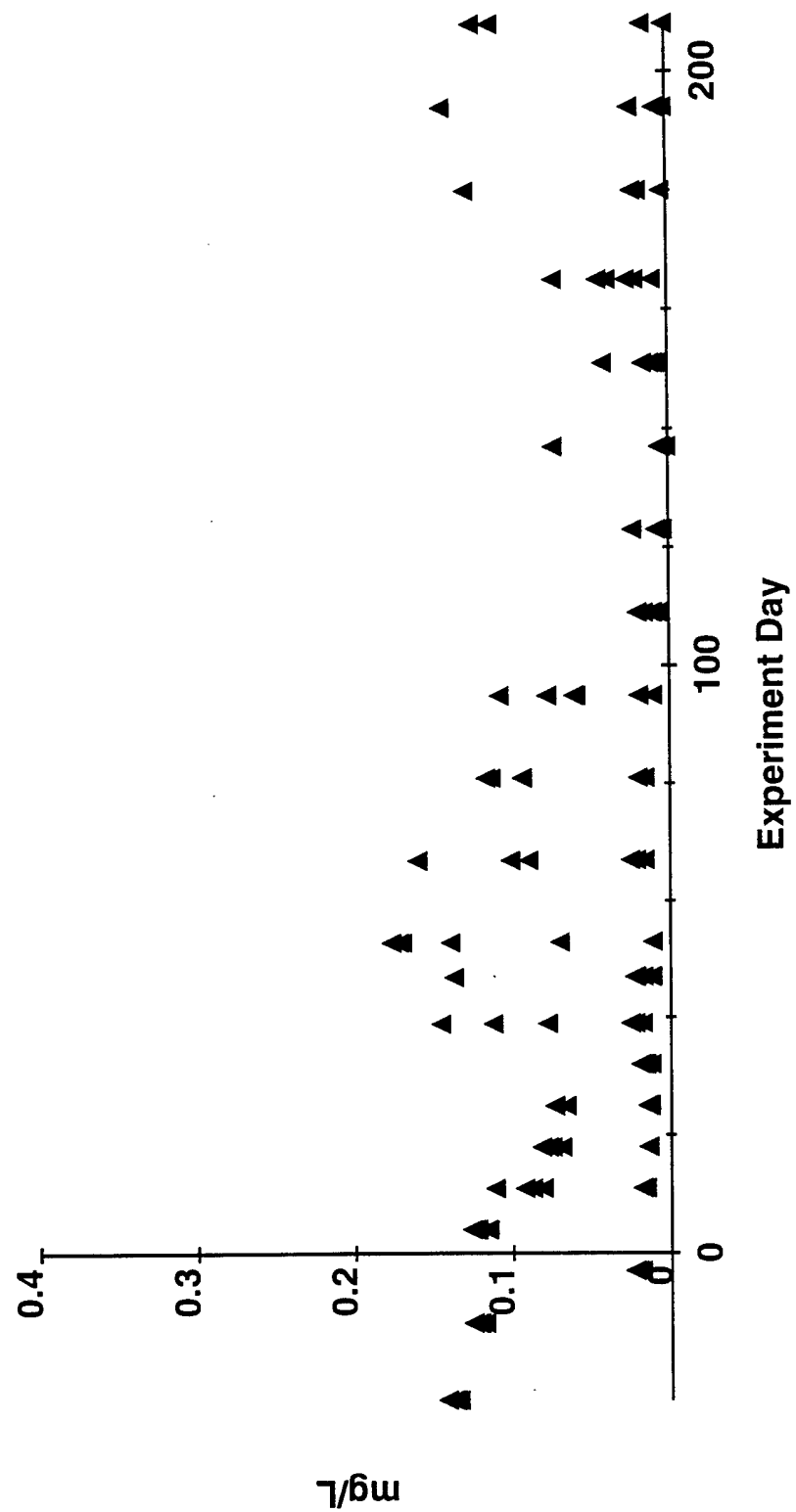
Figure 78c. Phytoplankton
Chlorophyll - Low Dose

Figure 78d. Phytoplankton
Chlorophyll - Undosed

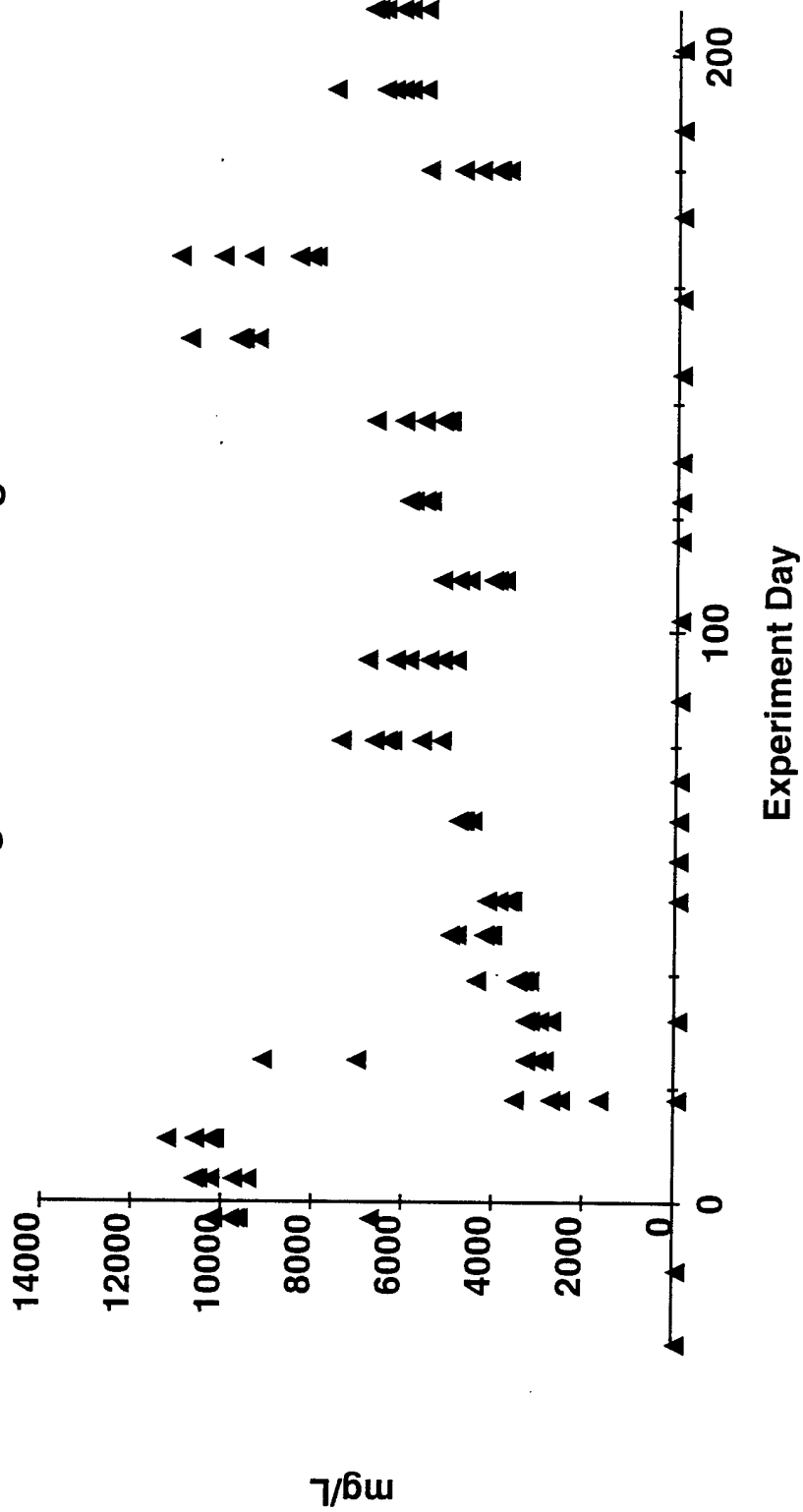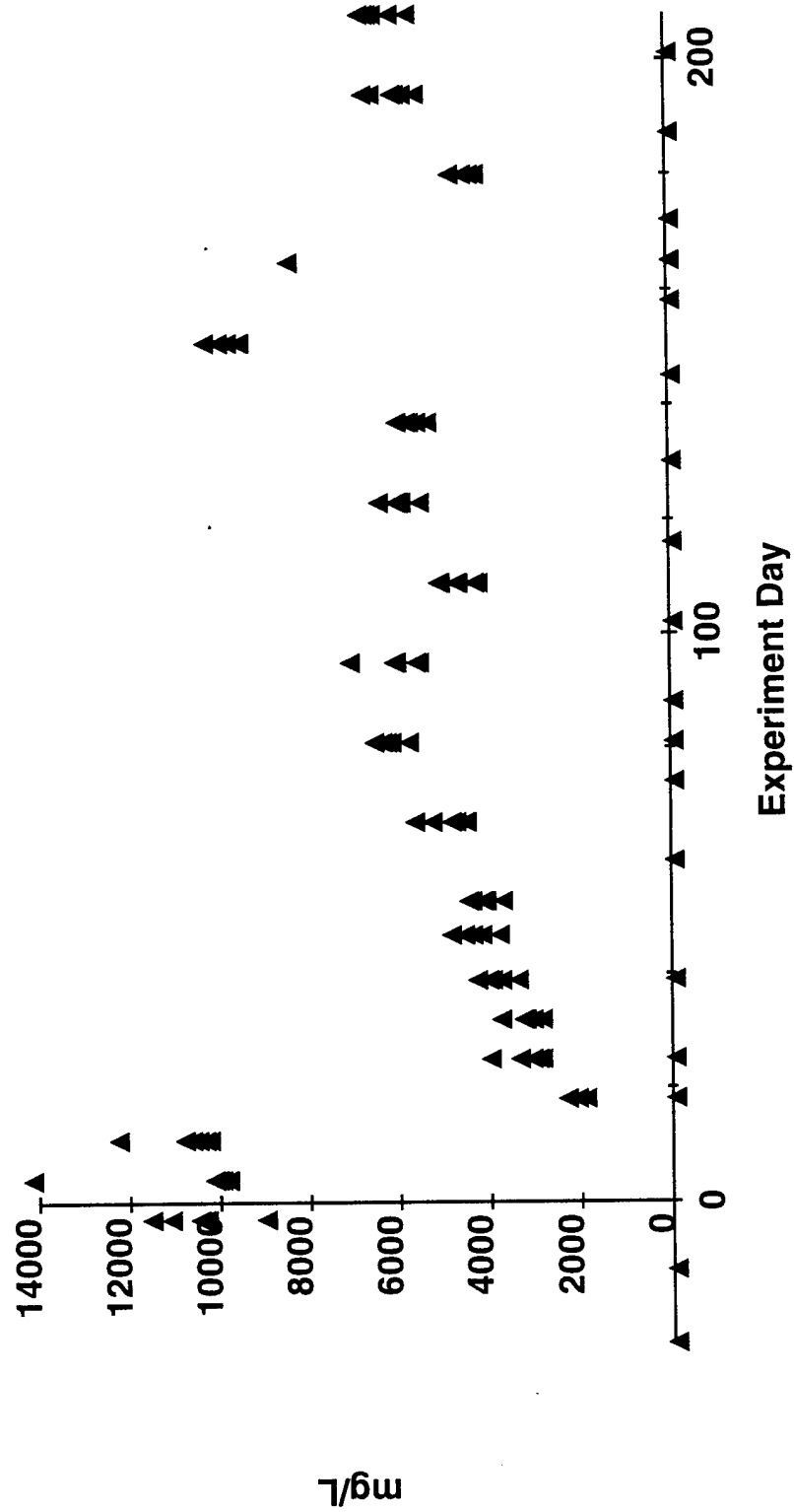Figure 79a. Phytoplankton
Total Organic Carbon - High Dose
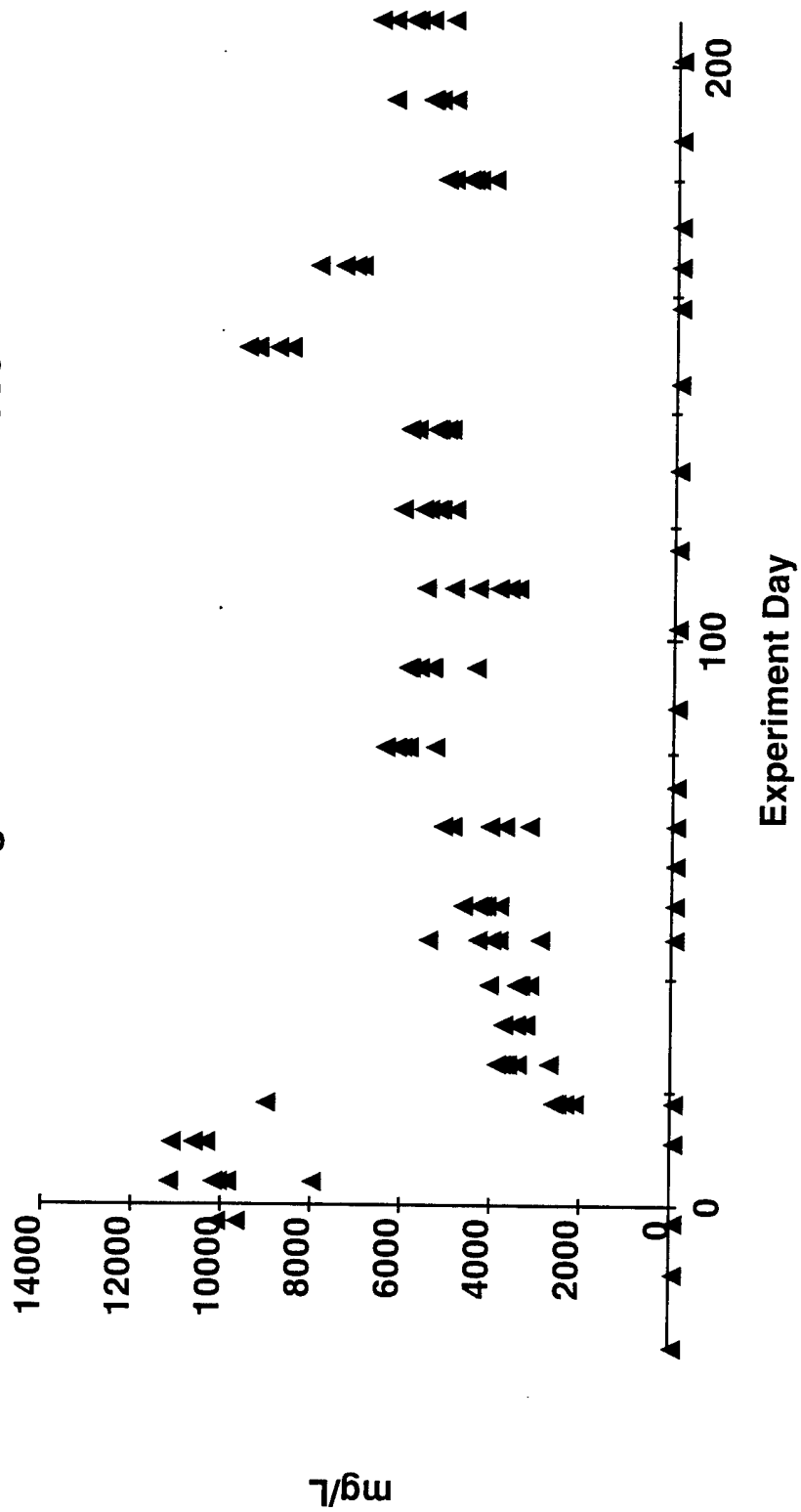
Figure 79b. Phytoplankton
Total Organic Carbon - Mid-dose

Figure 79c. Phytoplankton
Total Organic Carbon - Low Dose

Figure 79d. Phytoplankton
Total Organic Carbon - Undosed
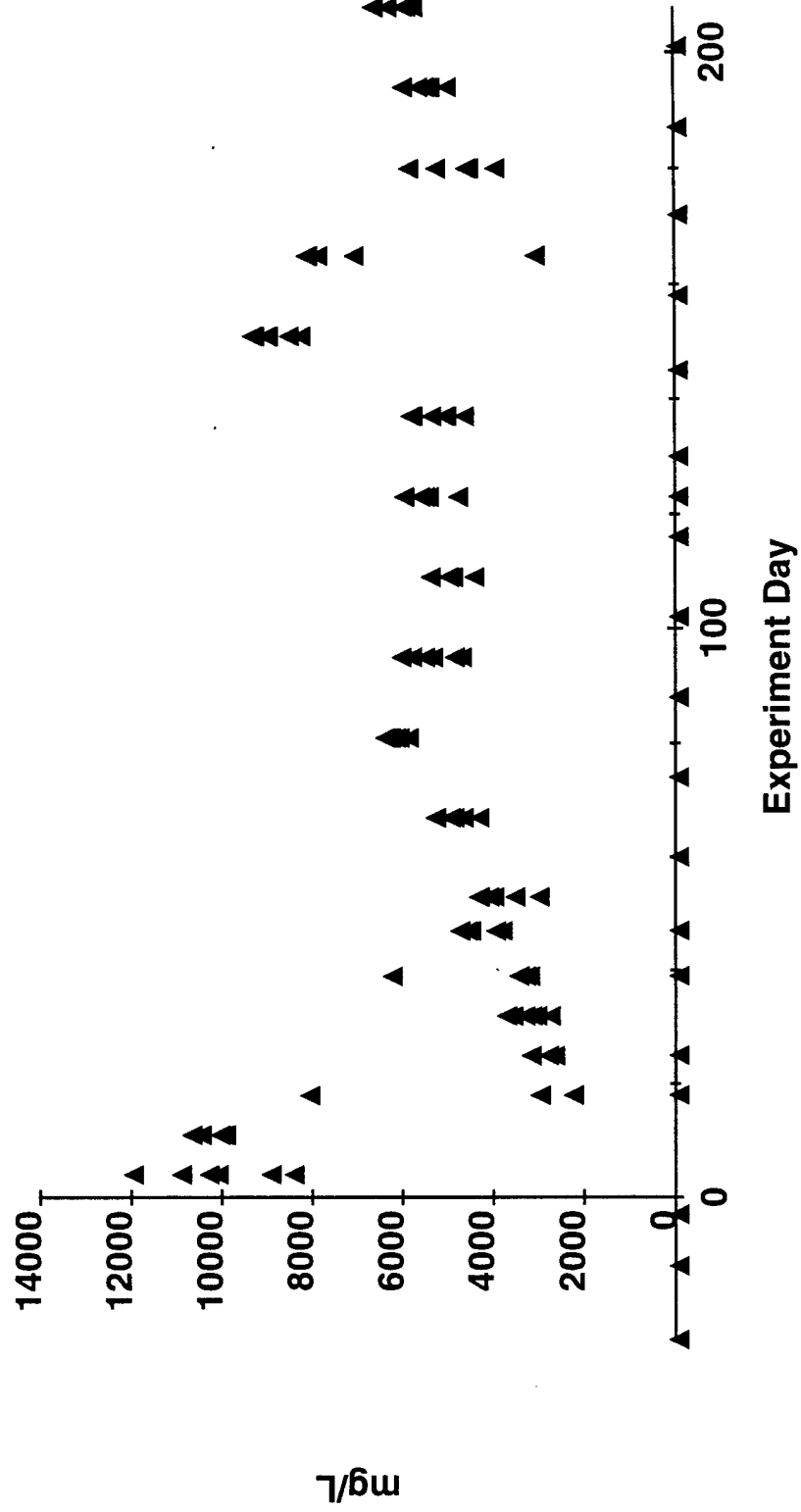
Figure 80a. Phytoplankton
Total Solids - High Dose
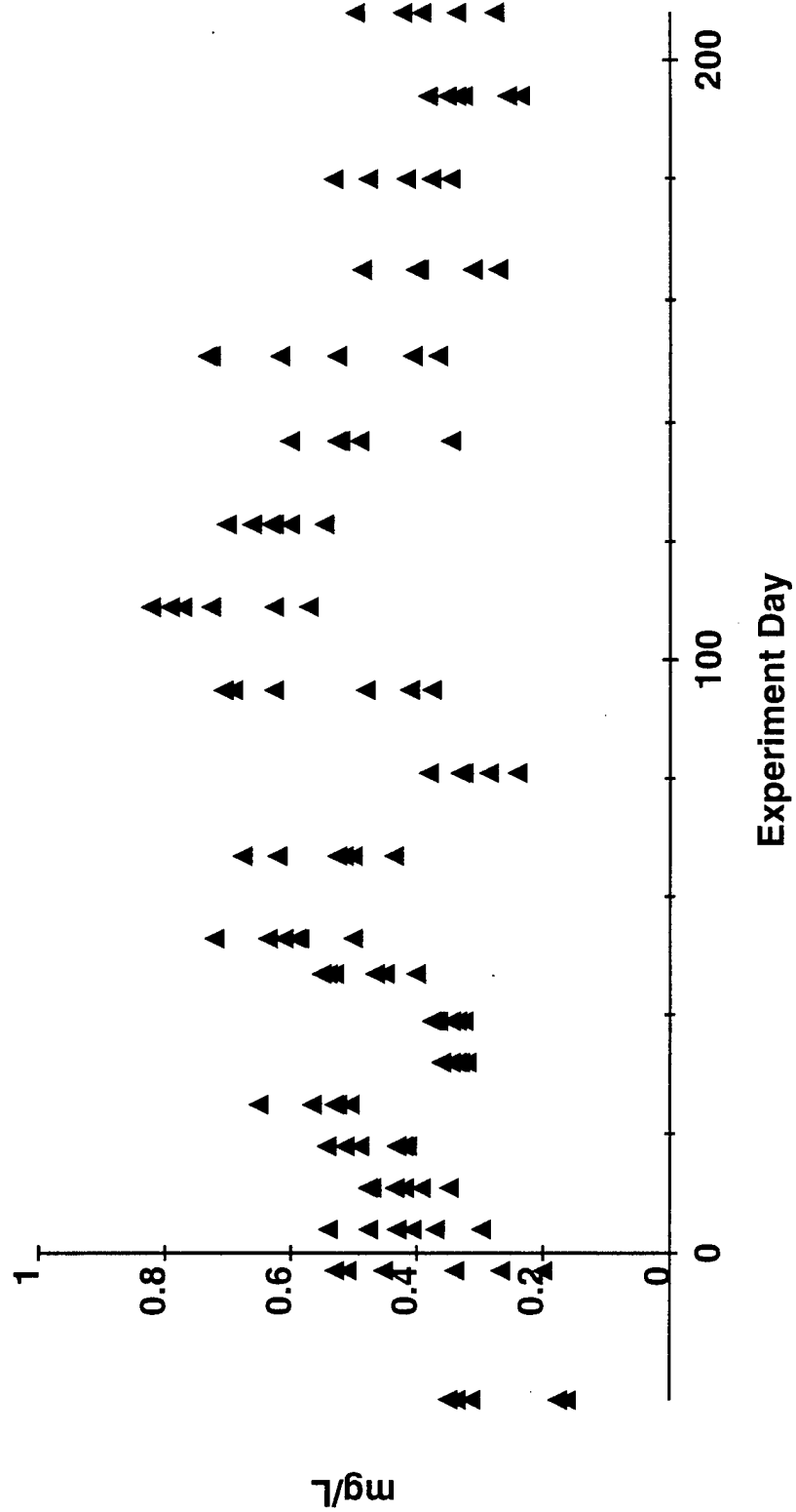
Figure 80b. Phytoplankton
Total Solids - Mid-dose
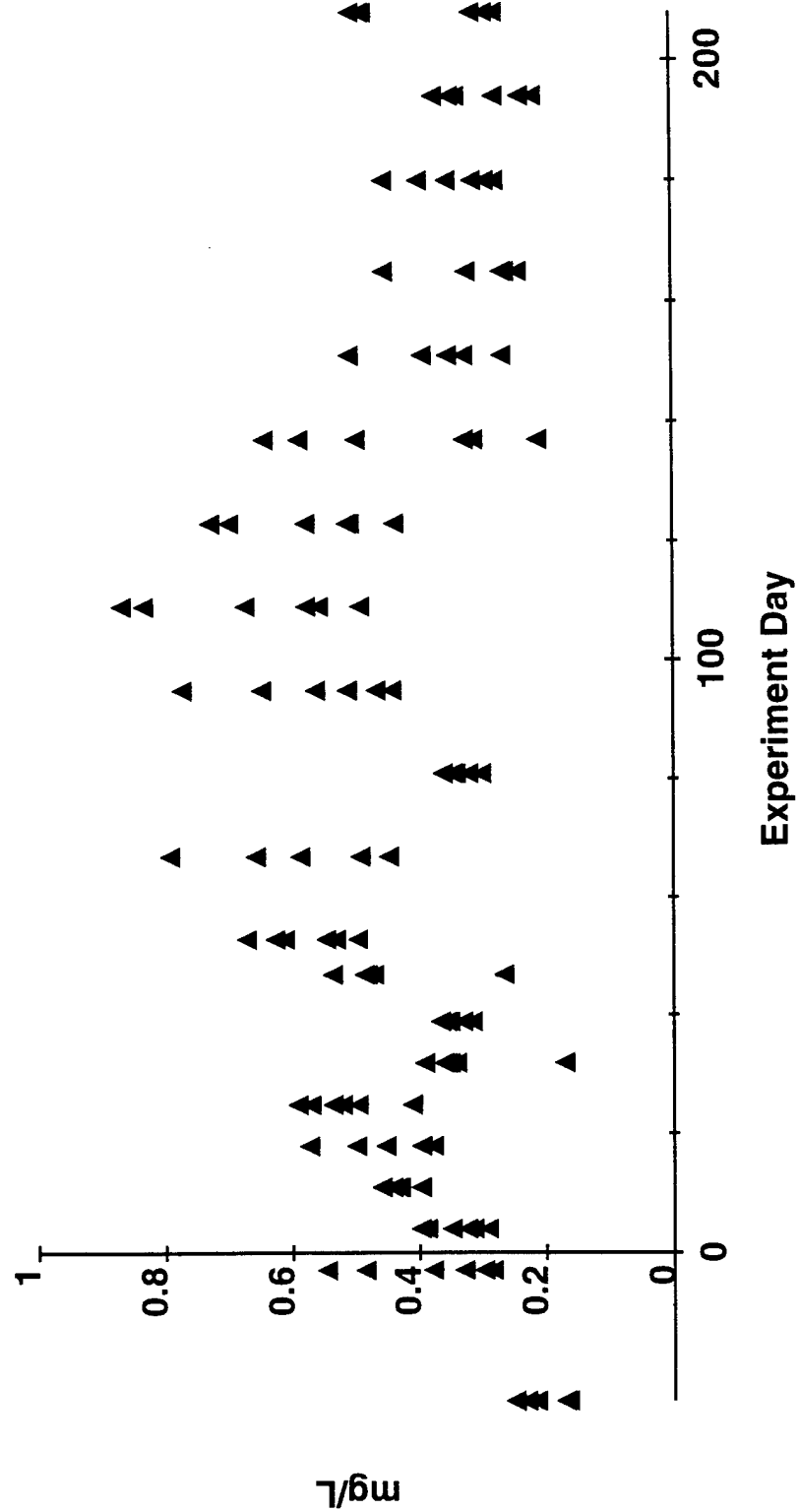
Figure 80c. Phytoplankton
Total Solids - Low Dose
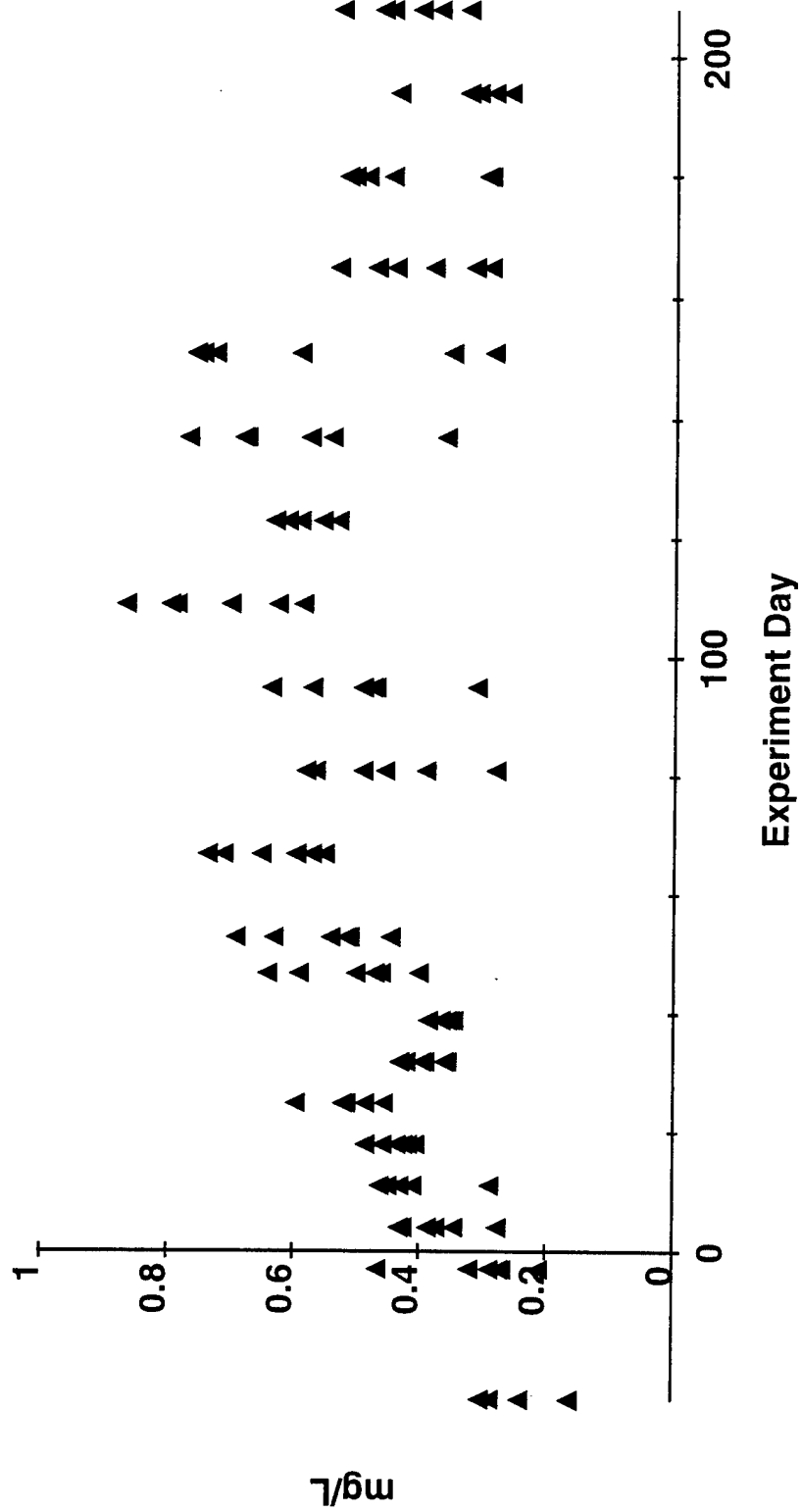
Figure 80d. Phytoplankton
Total Solids - Undosed
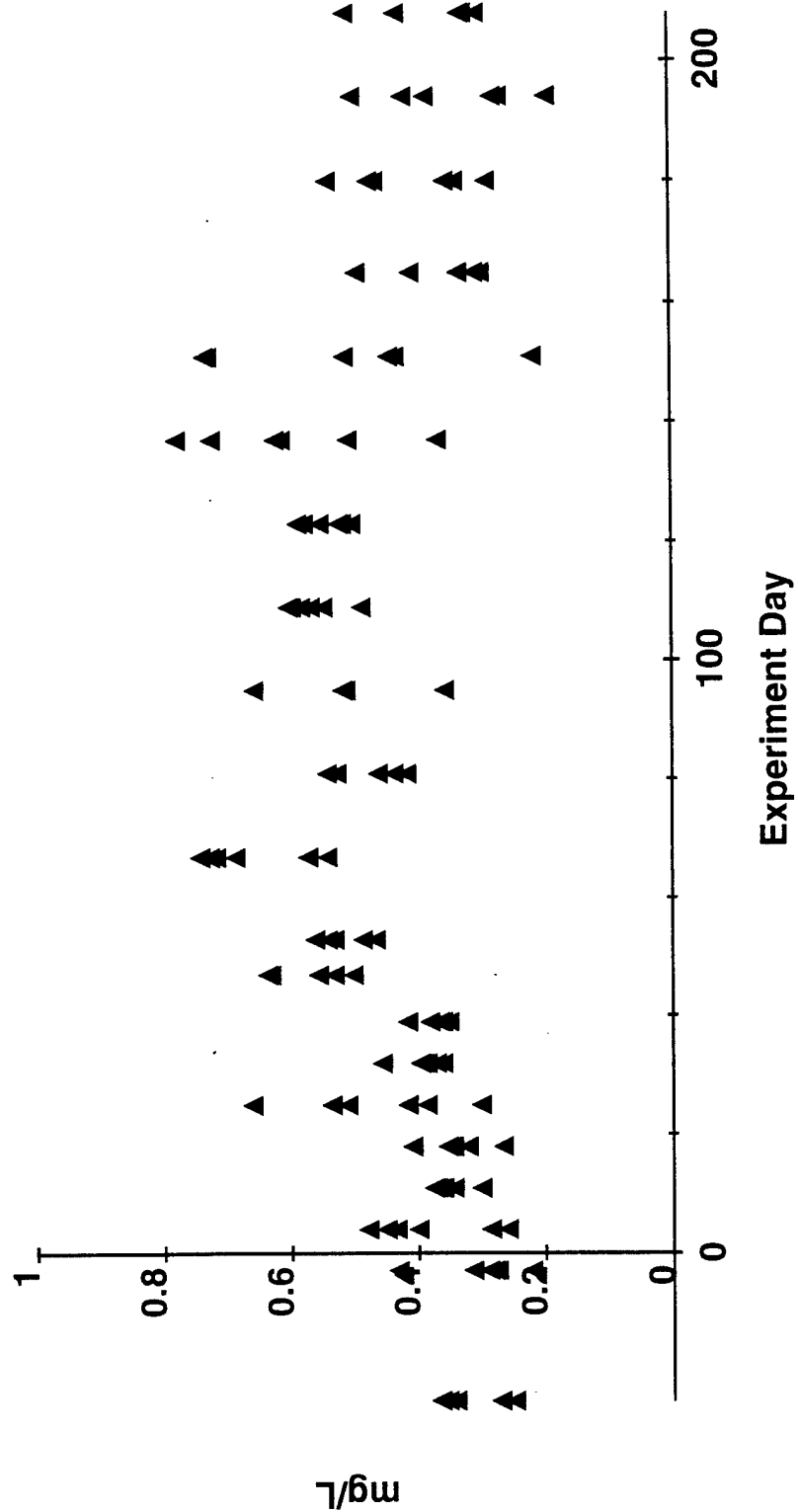
Figure 81a. Phytoplankton
Total Volatile Solids - High Dose
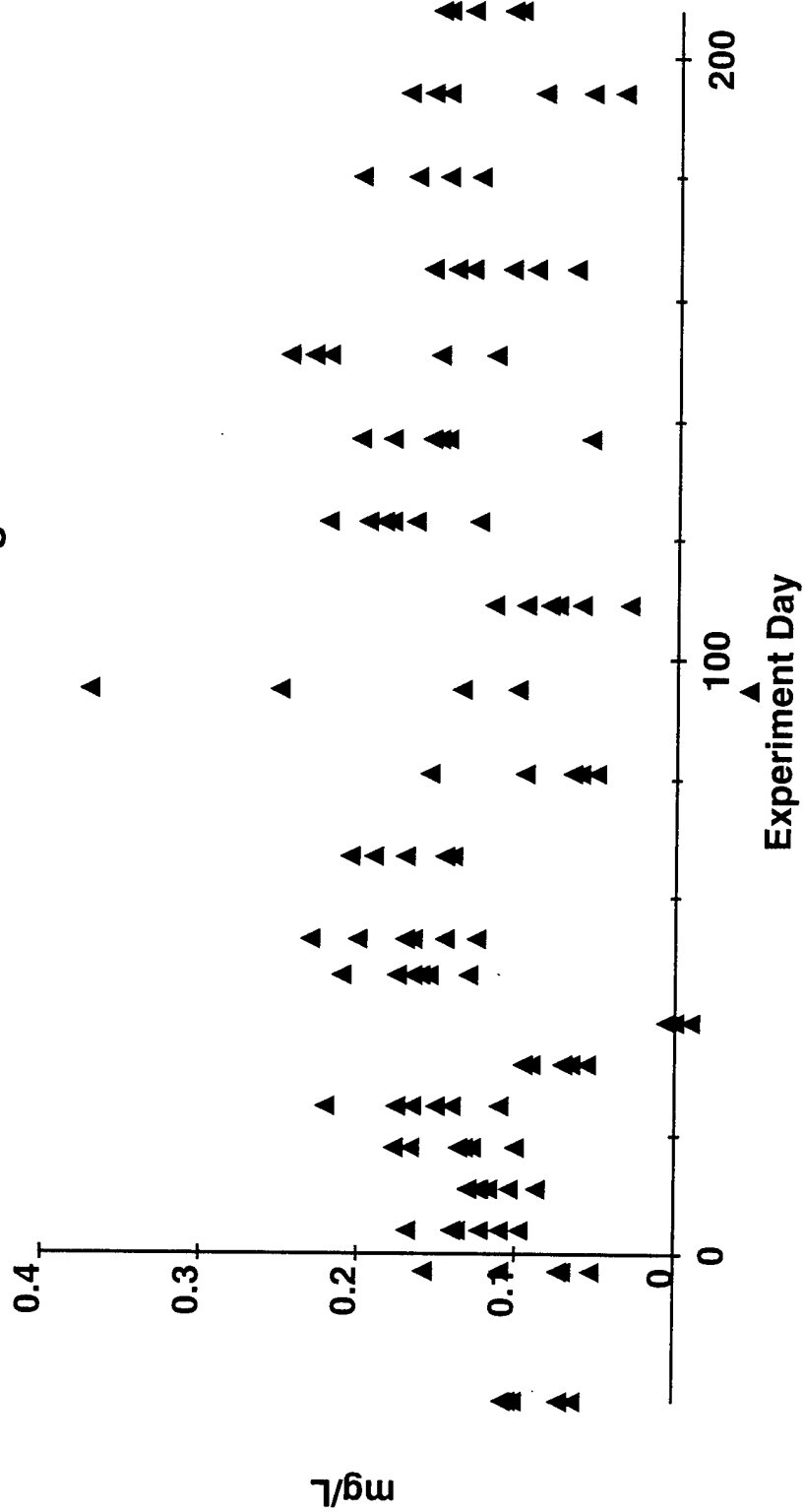
Figure 81b. Phytoplankton
Total Volatile Solids - Mid-dose
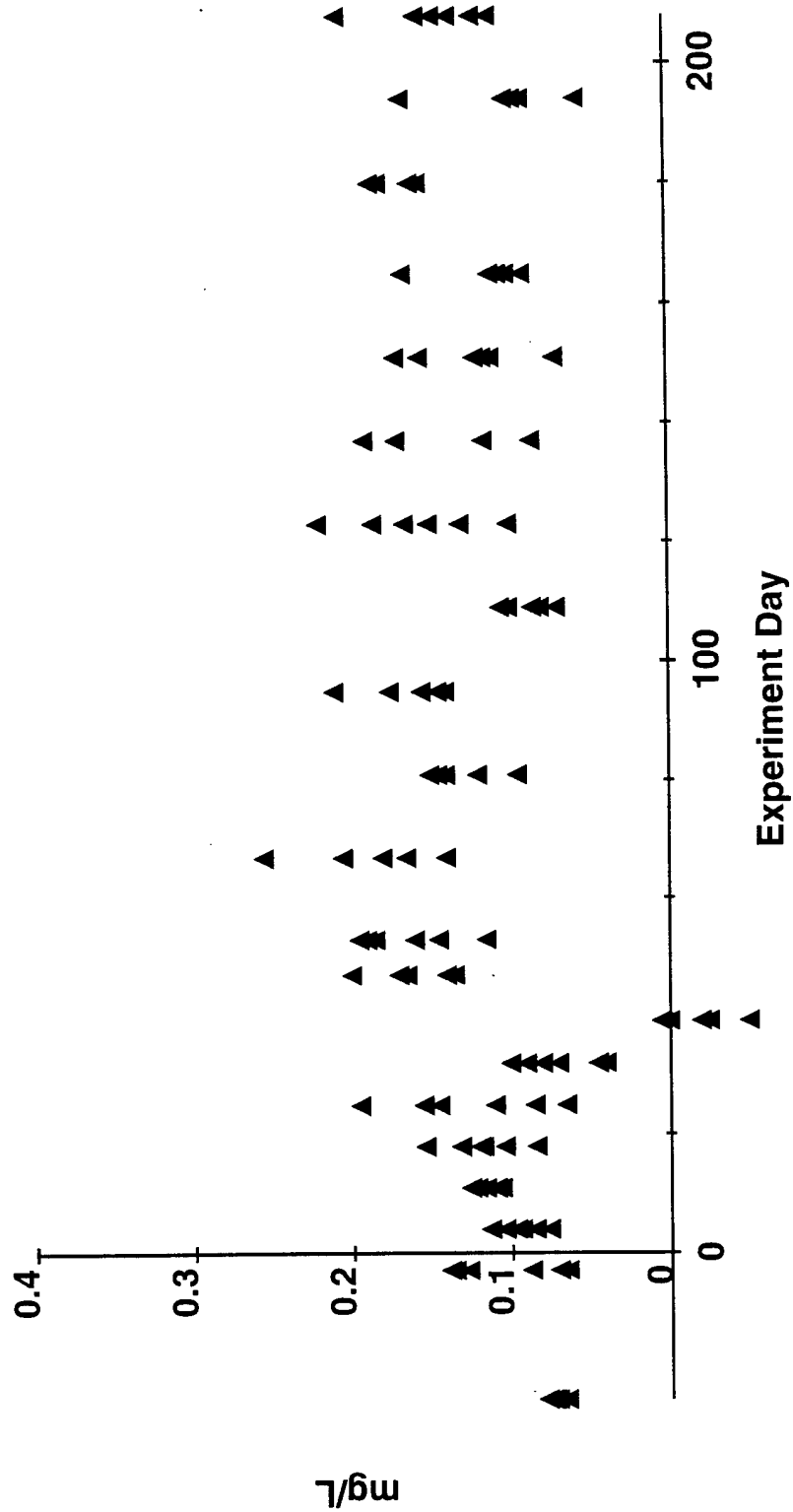
Figure 81c. Phytoplankton
Total Volatile Solids - Low Dose
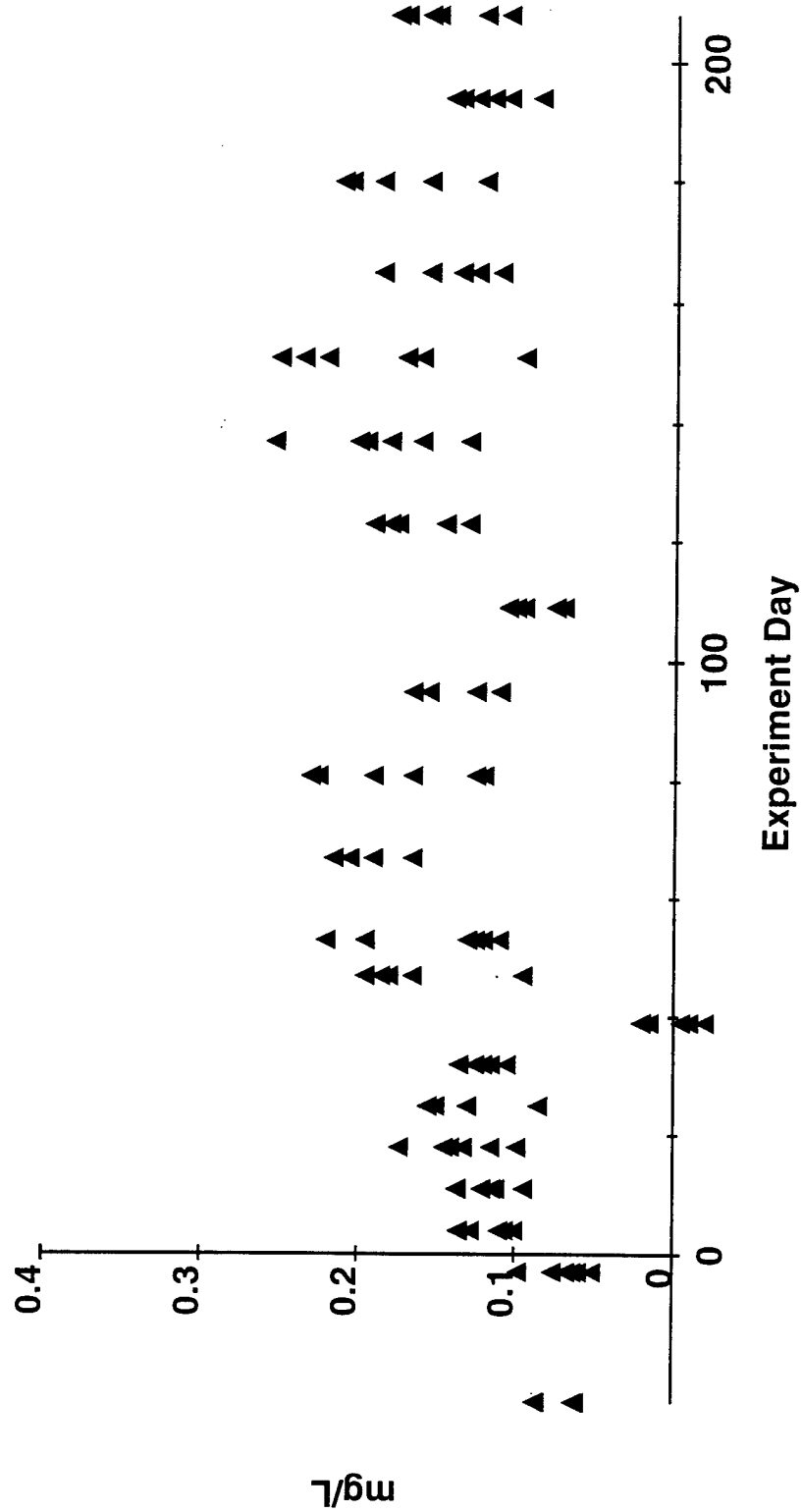
Figure 81d. Phytoplankton
Total Volatile Solids - Undosed
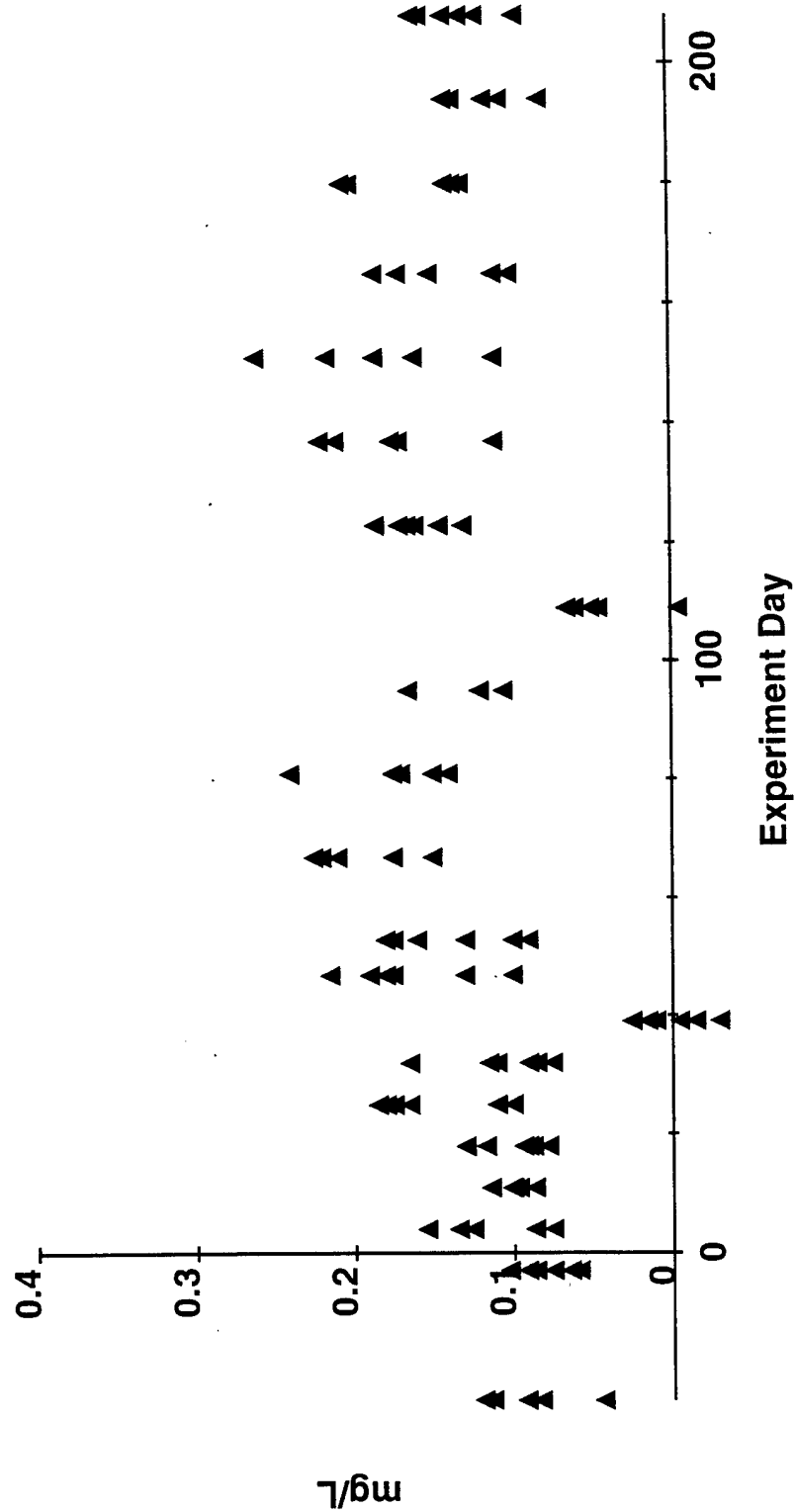
Figure 82a. Phytoplankton
TTC Respiration - High Dose
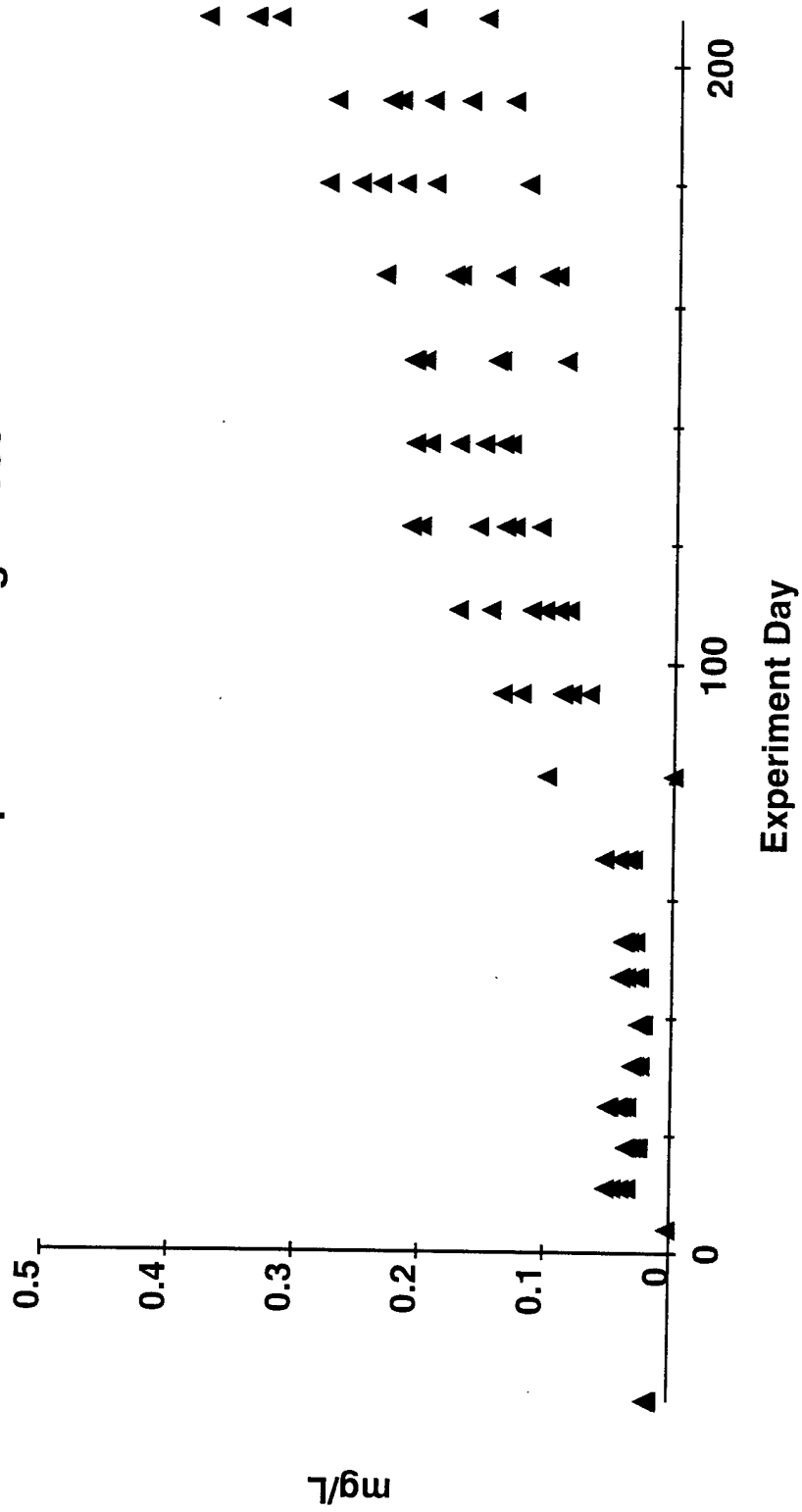
Figure 82b. Phytoplankton
TTC Respiration - Mid-dose
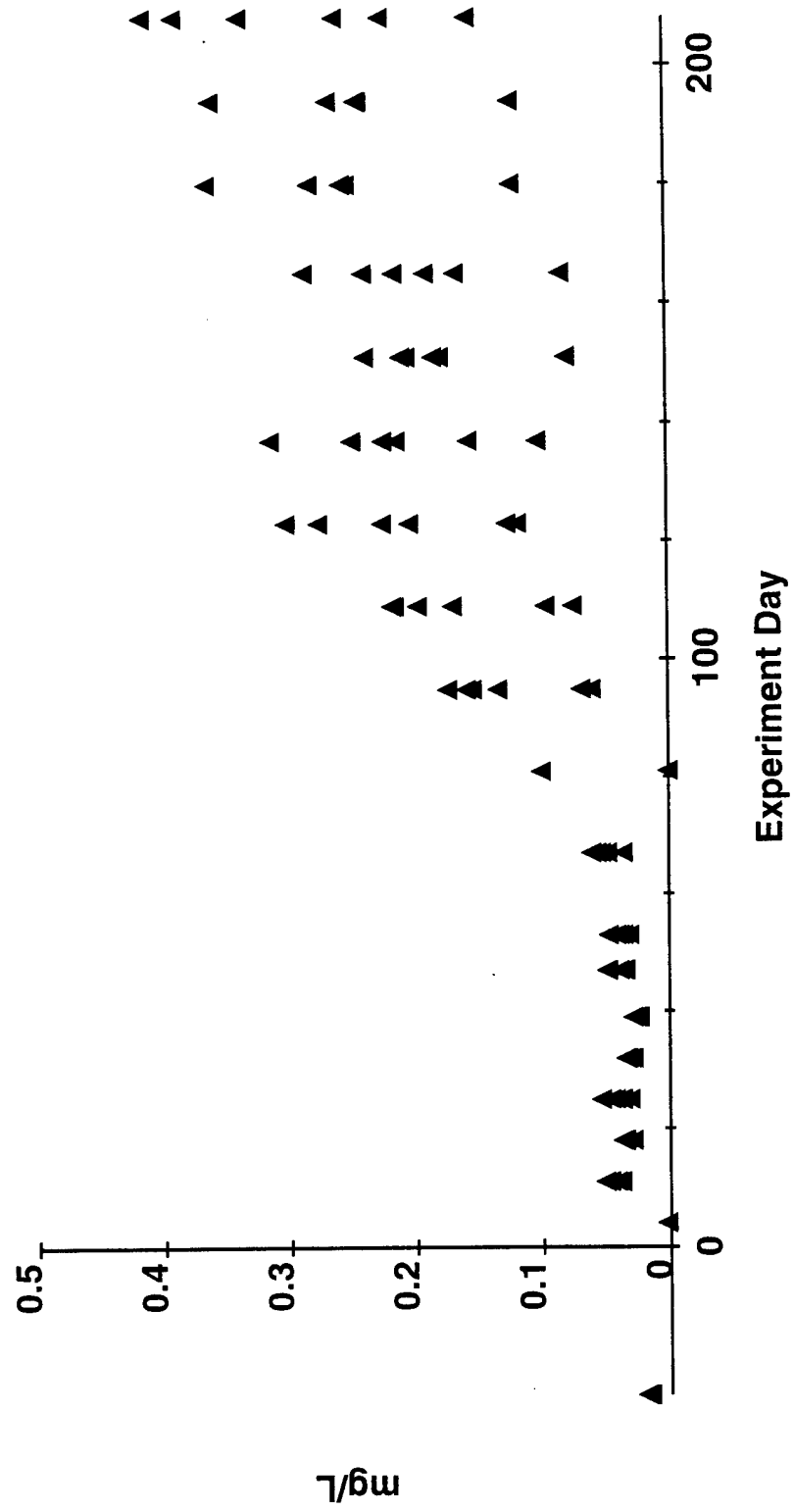
Figure 82c. Phytoplankton
TTC Respiration - Low Dose
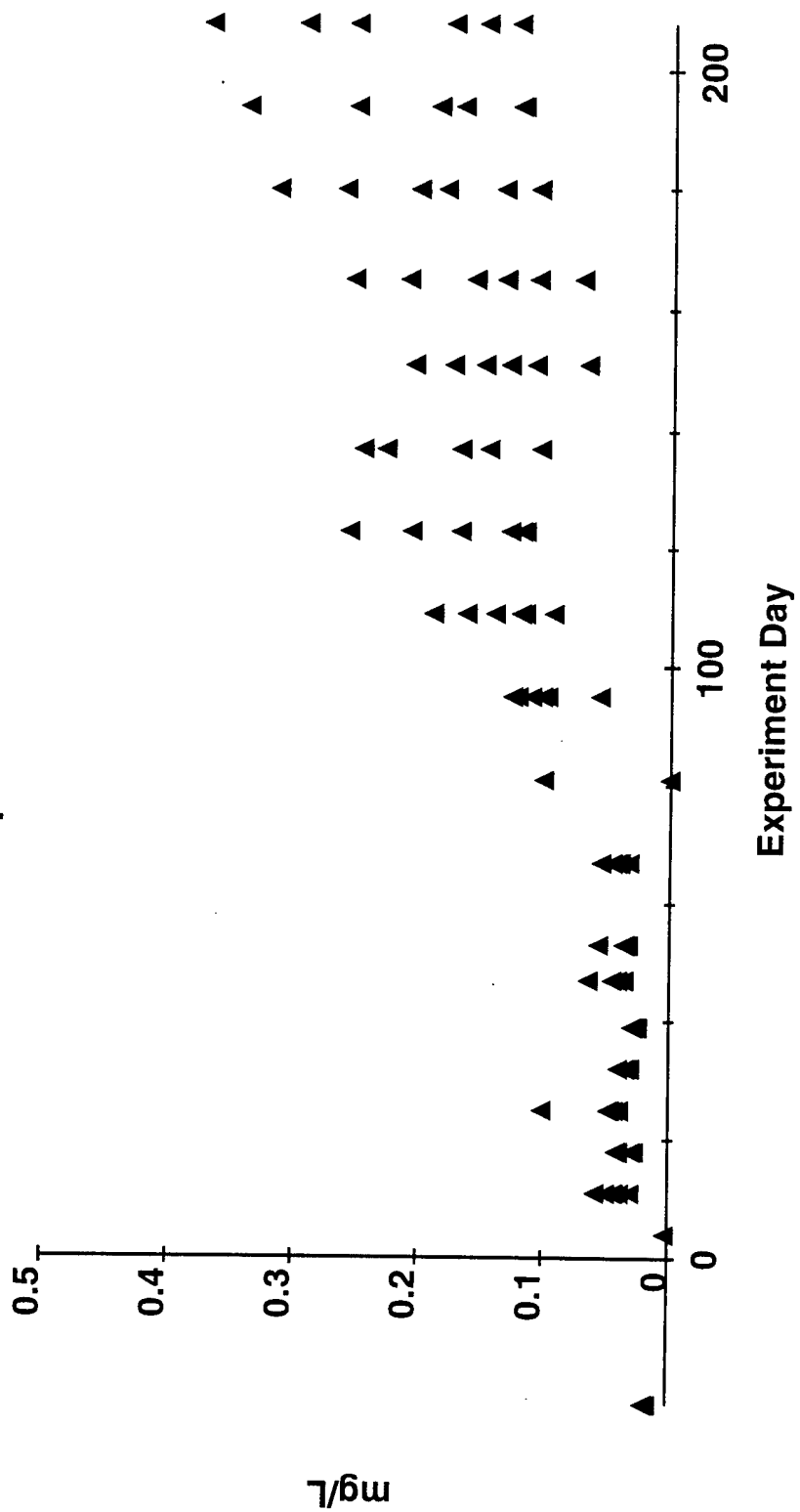
Figure 82d. Phytoplankton
TTC Respiration - Undosed
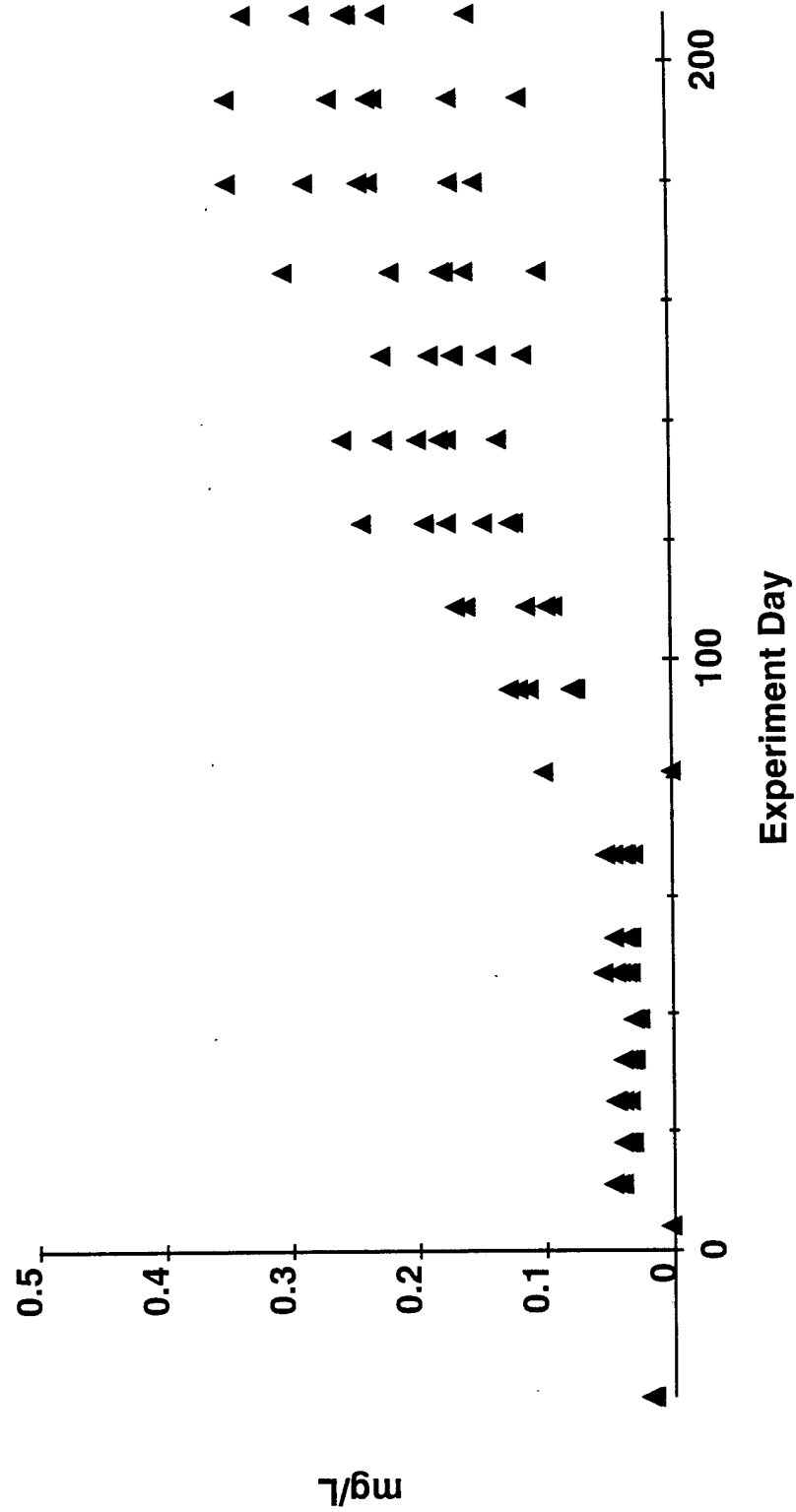
Figure 83a. Water Quality
Alkalinity - High Dose
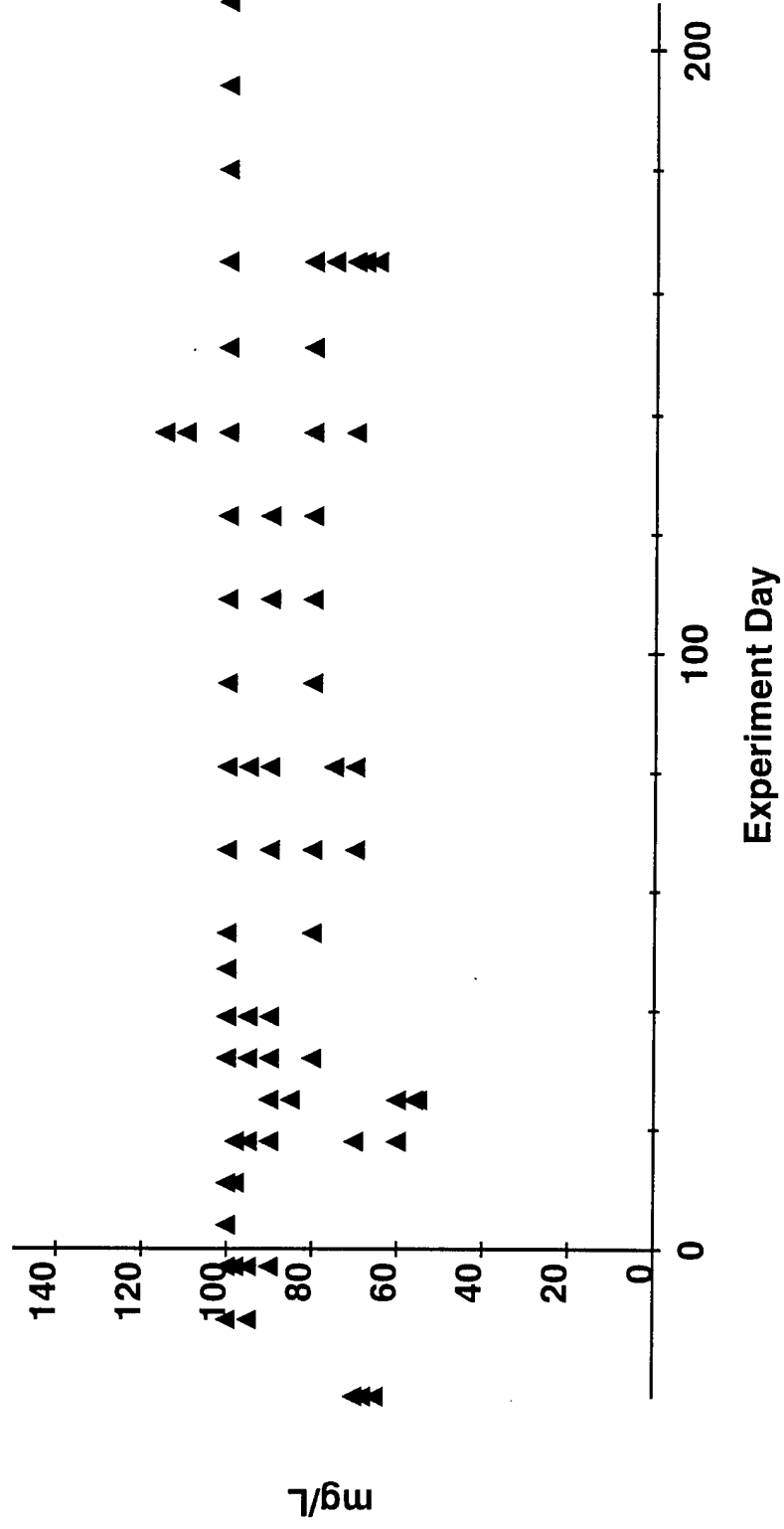
Figure 83b. Water Quality
Alkalinity - Mid-dose
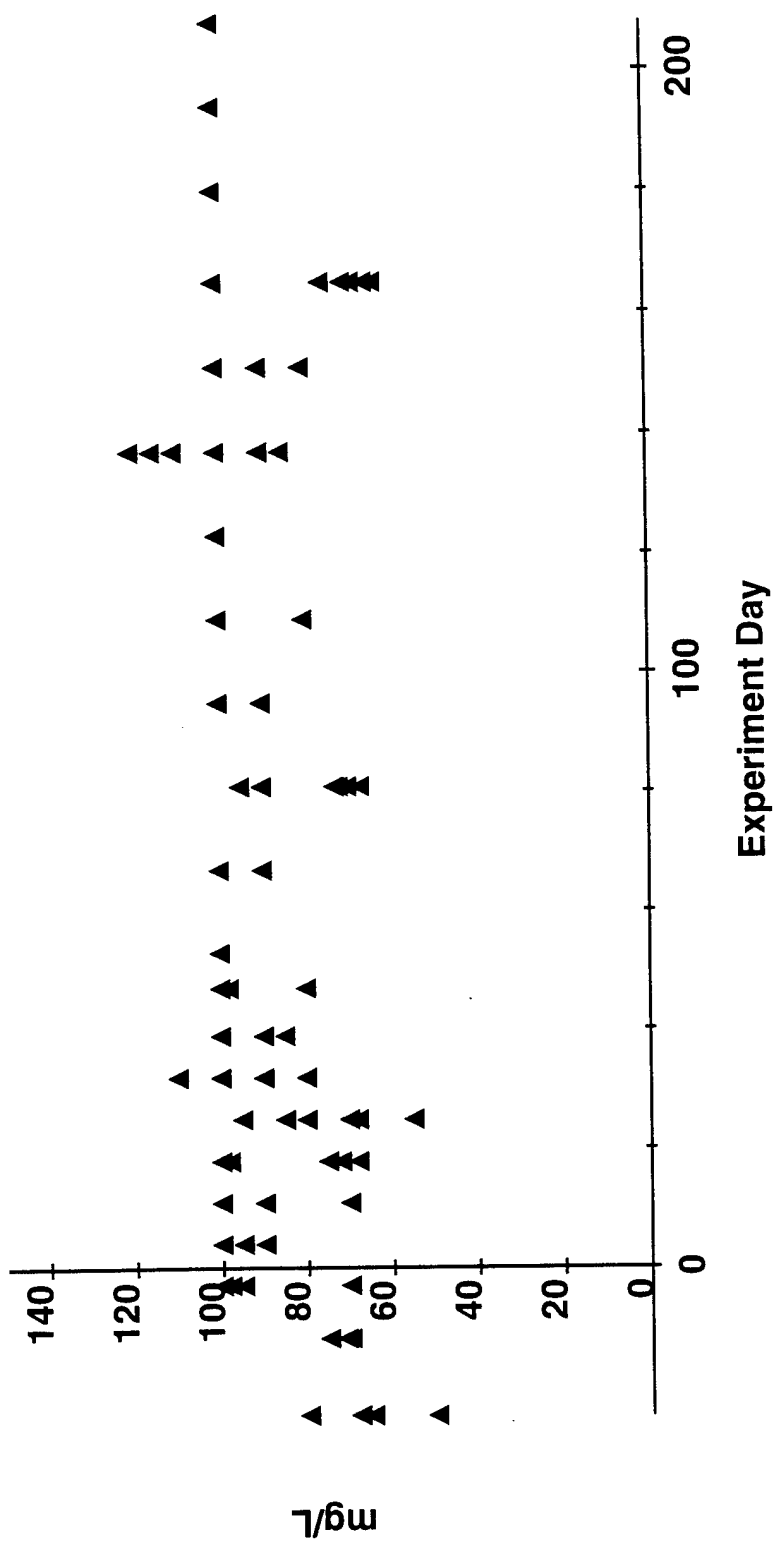
Figure 83c. Water Quality
Alkalinity - Low Dose
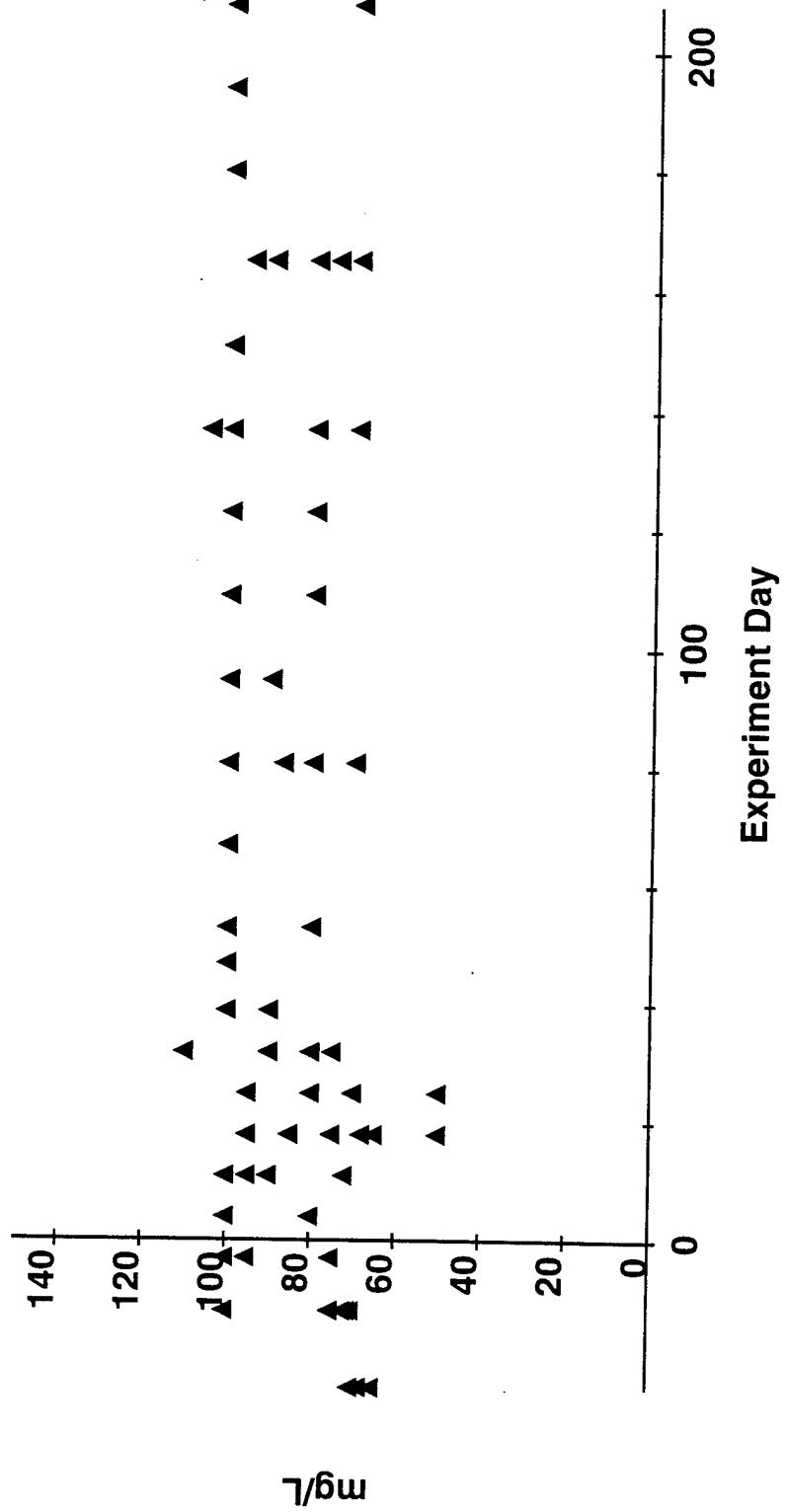
Figure 83d. Water Quality
Alkalinity - Undosed
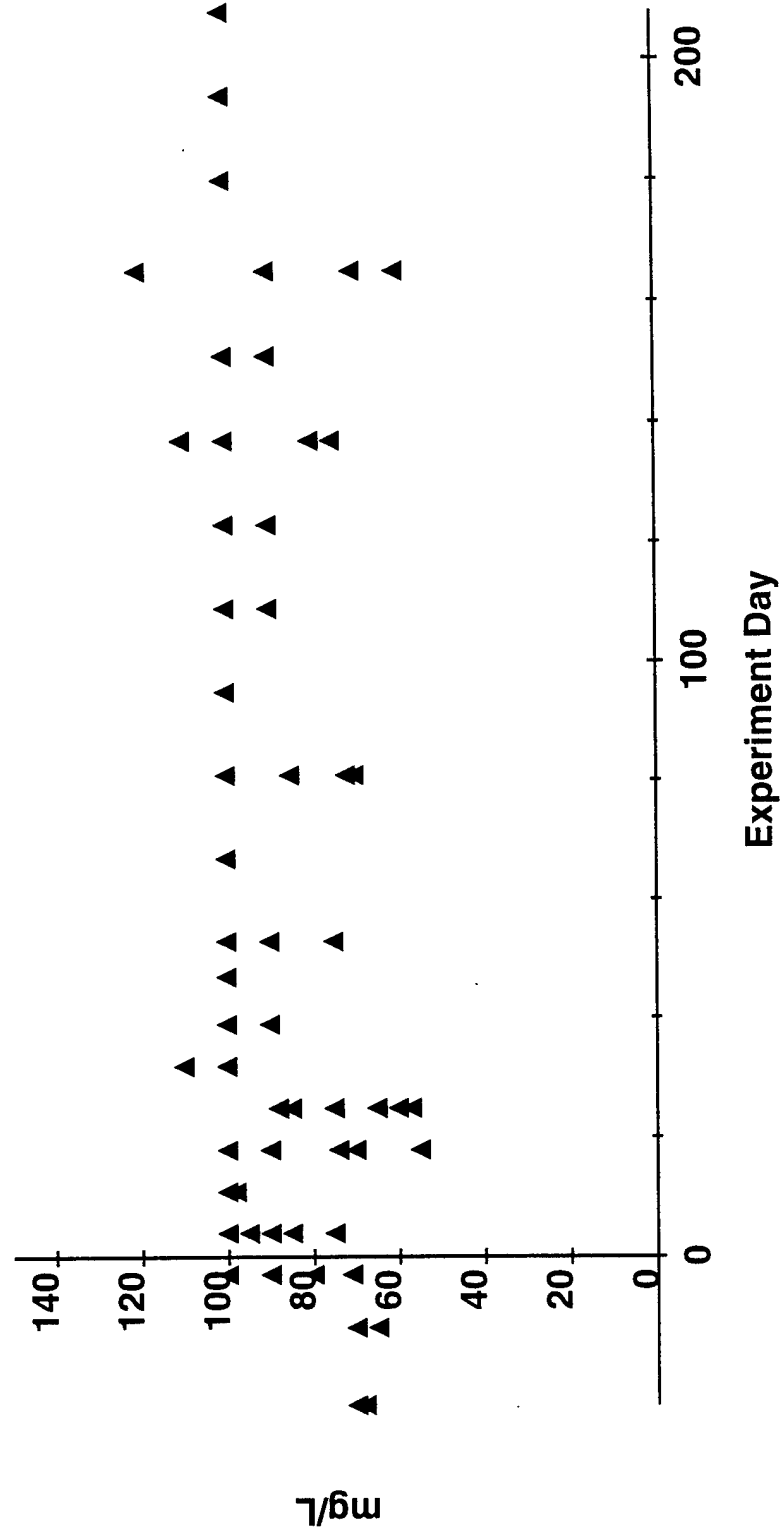
Figure 84a. Water Quality
Ambient Light - High Dose
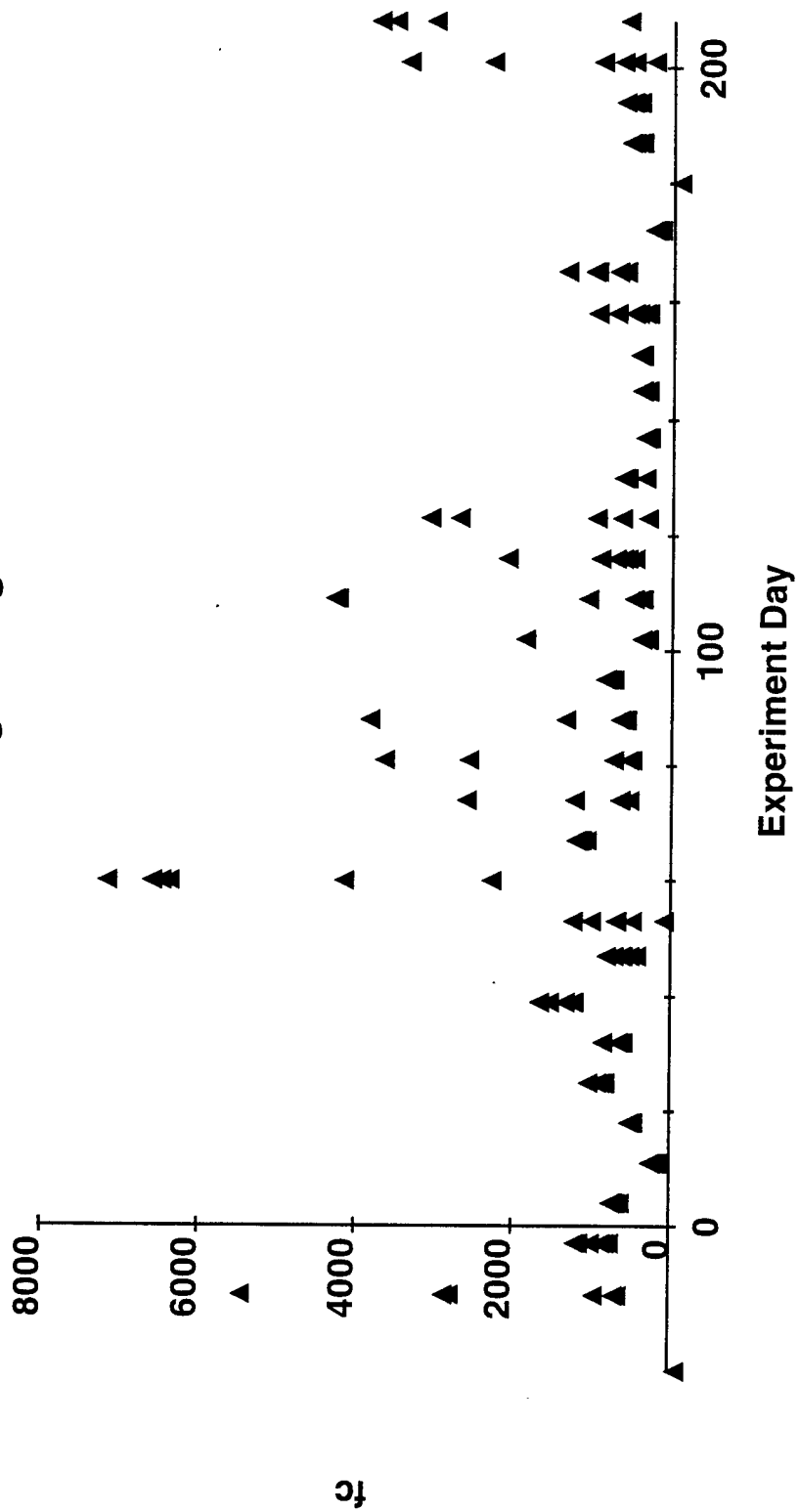
Figure 84b. Water Quality
Ambient Light - Mid-dose
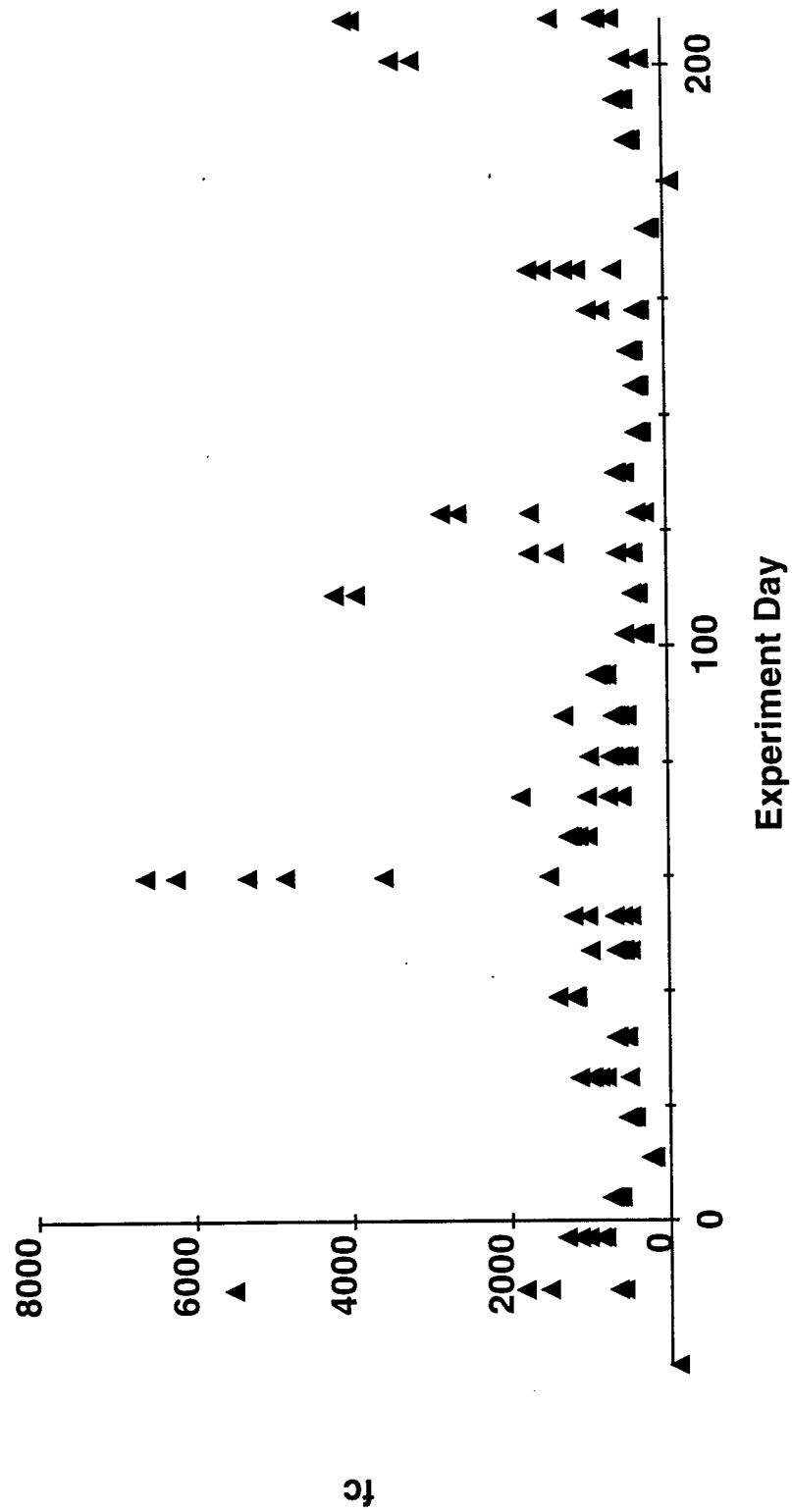
Figure 84c. Water Quality
Ambient Light - Low Dose

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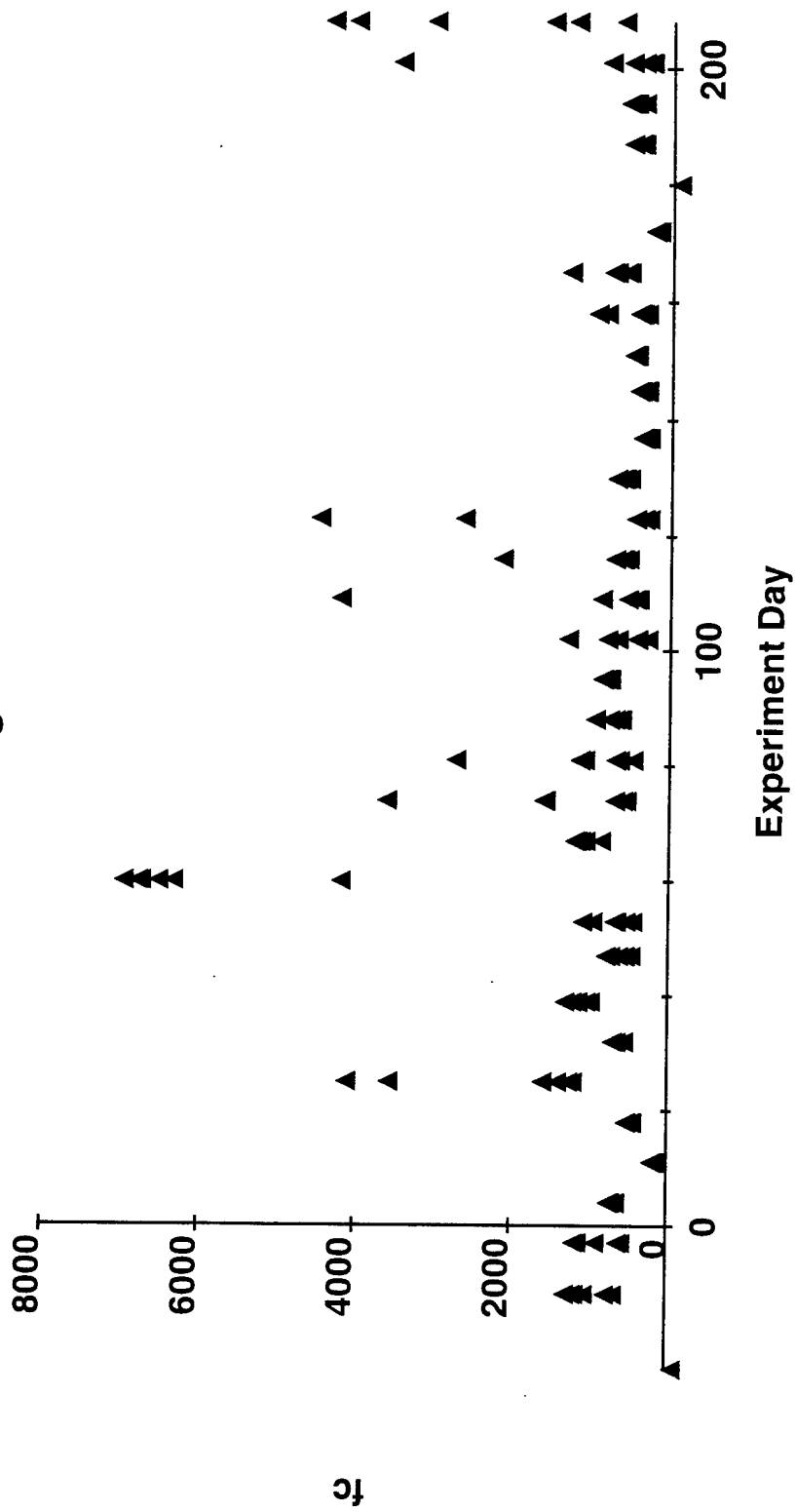
Figure 84d. Water Quality
Ambient Light - Undosed
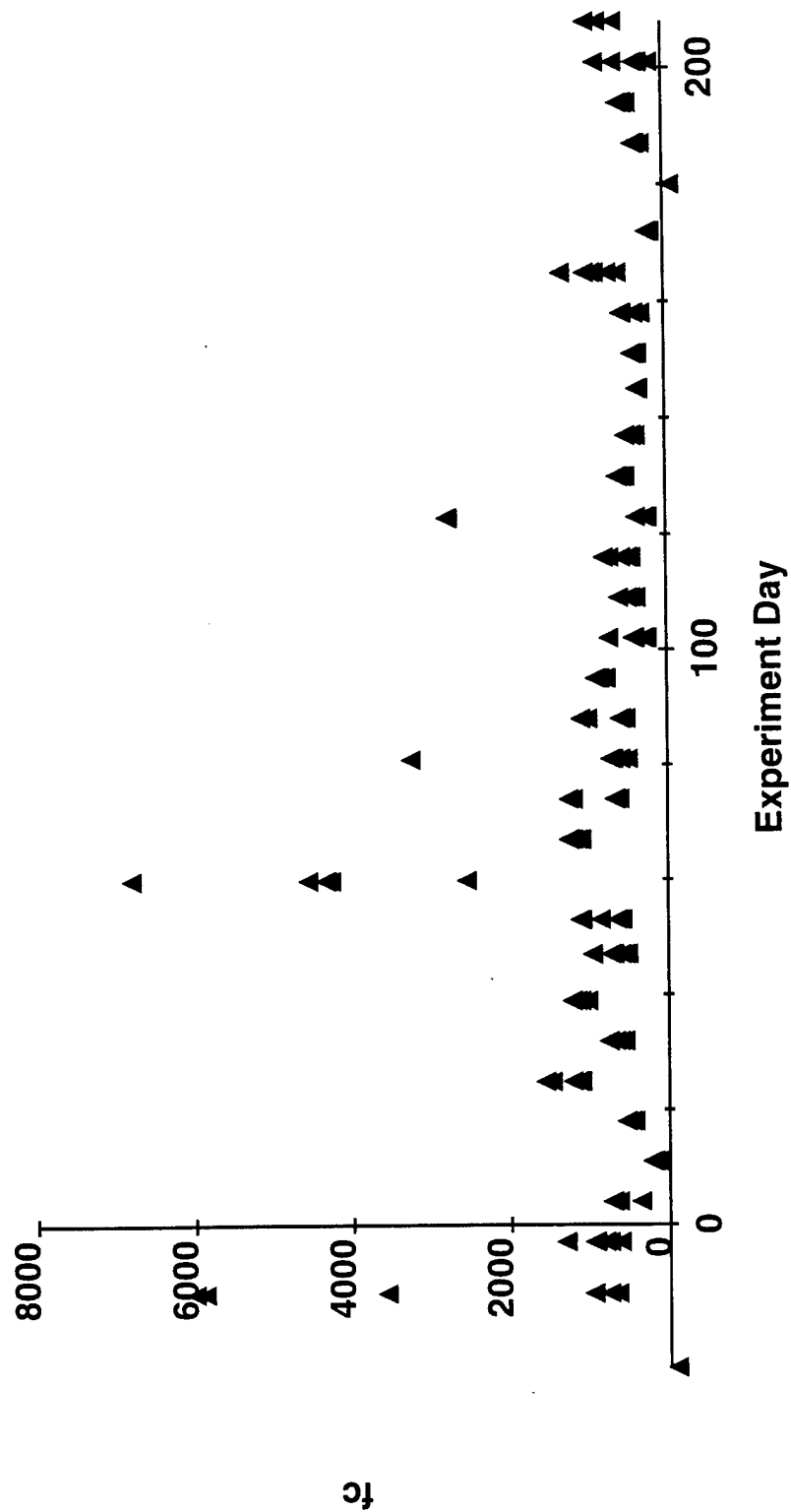
Figure 85a. Water Quality
Conductivity - High Dose
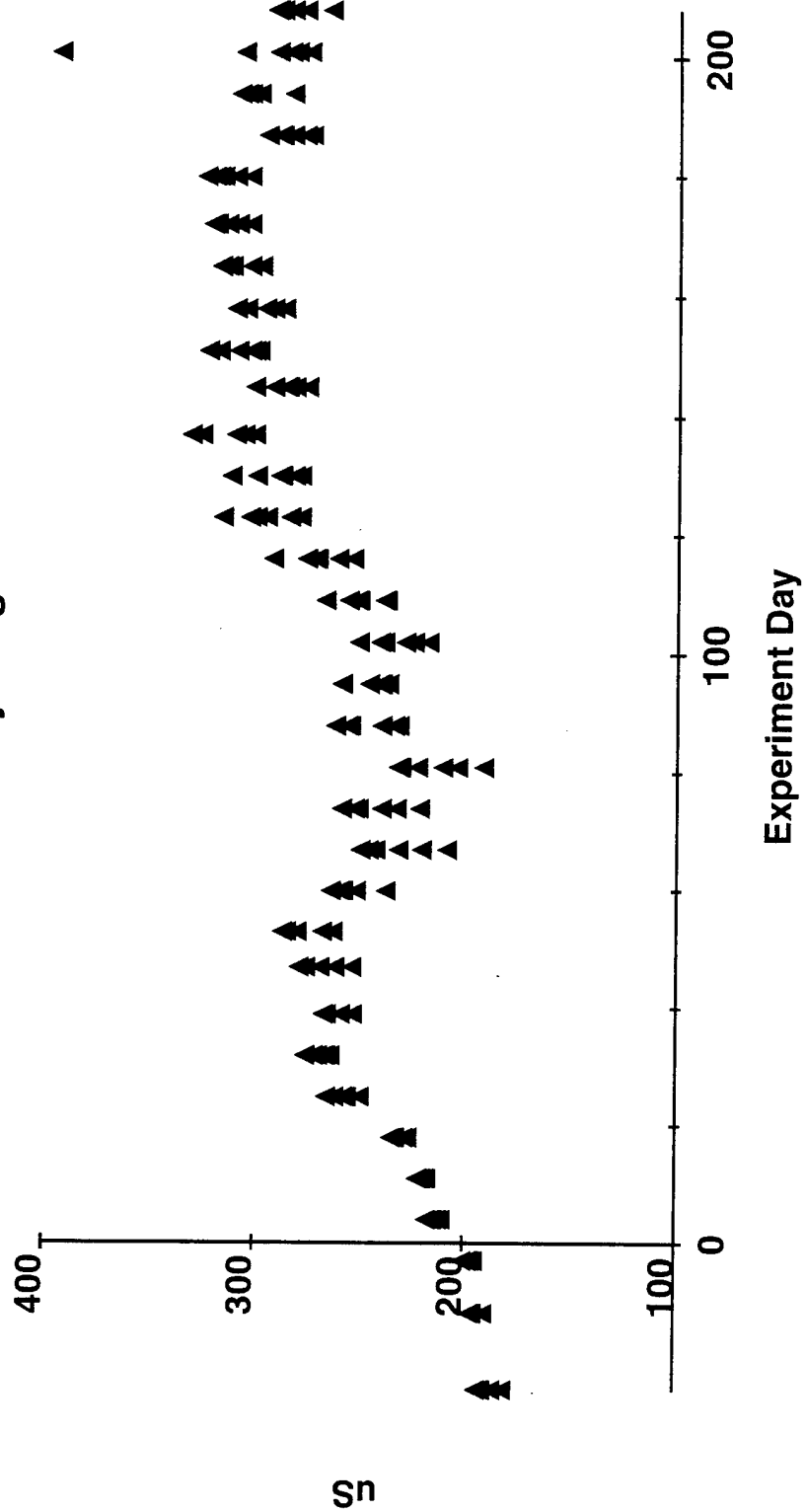
Figure 85b. Water Quality
Conductivity - Mid-dose
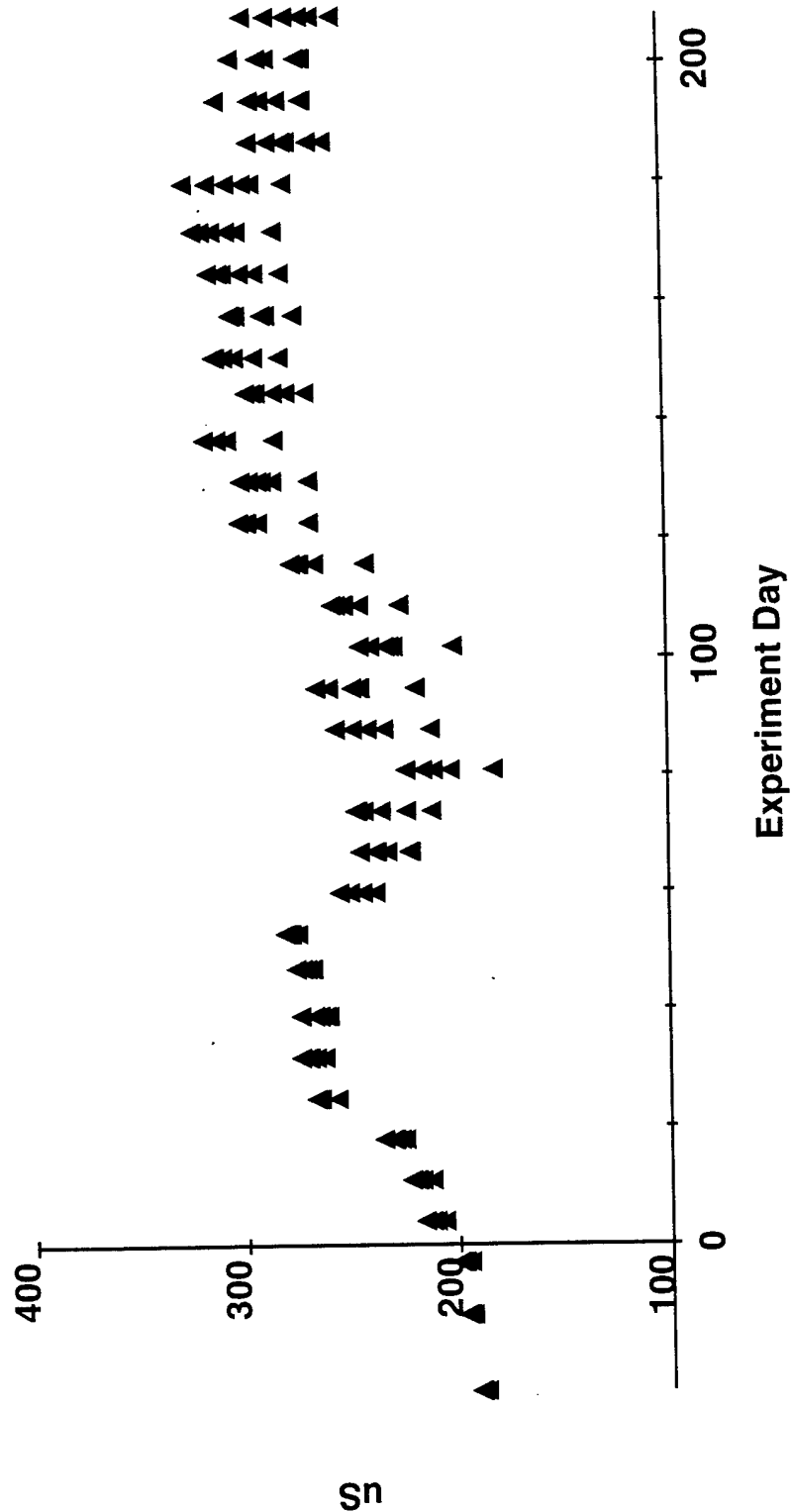
Figure 85c. Water Quality
Conductivity - Low Dose
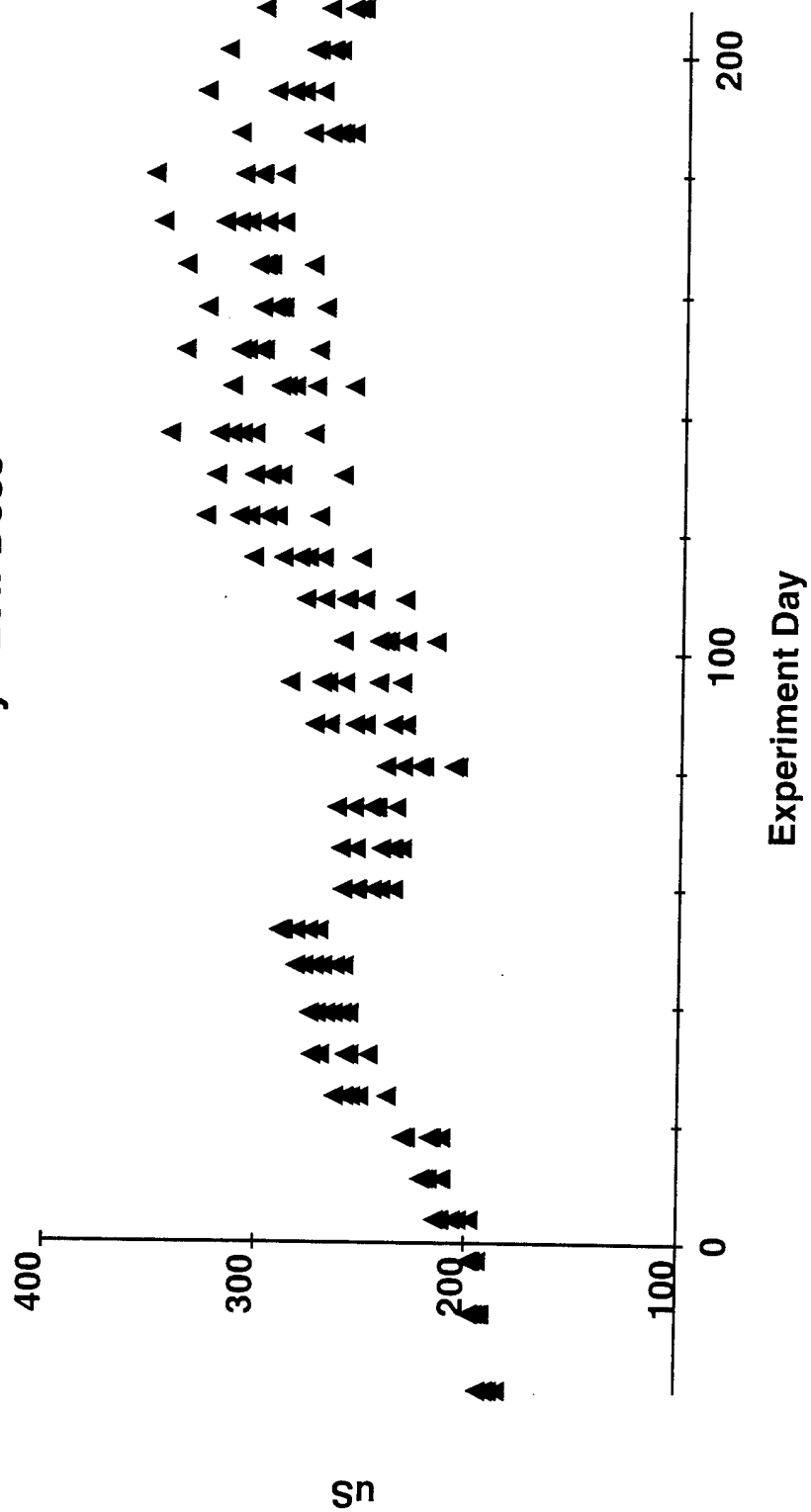
Figure 85d. Water Quality
Conductivity - Undosed
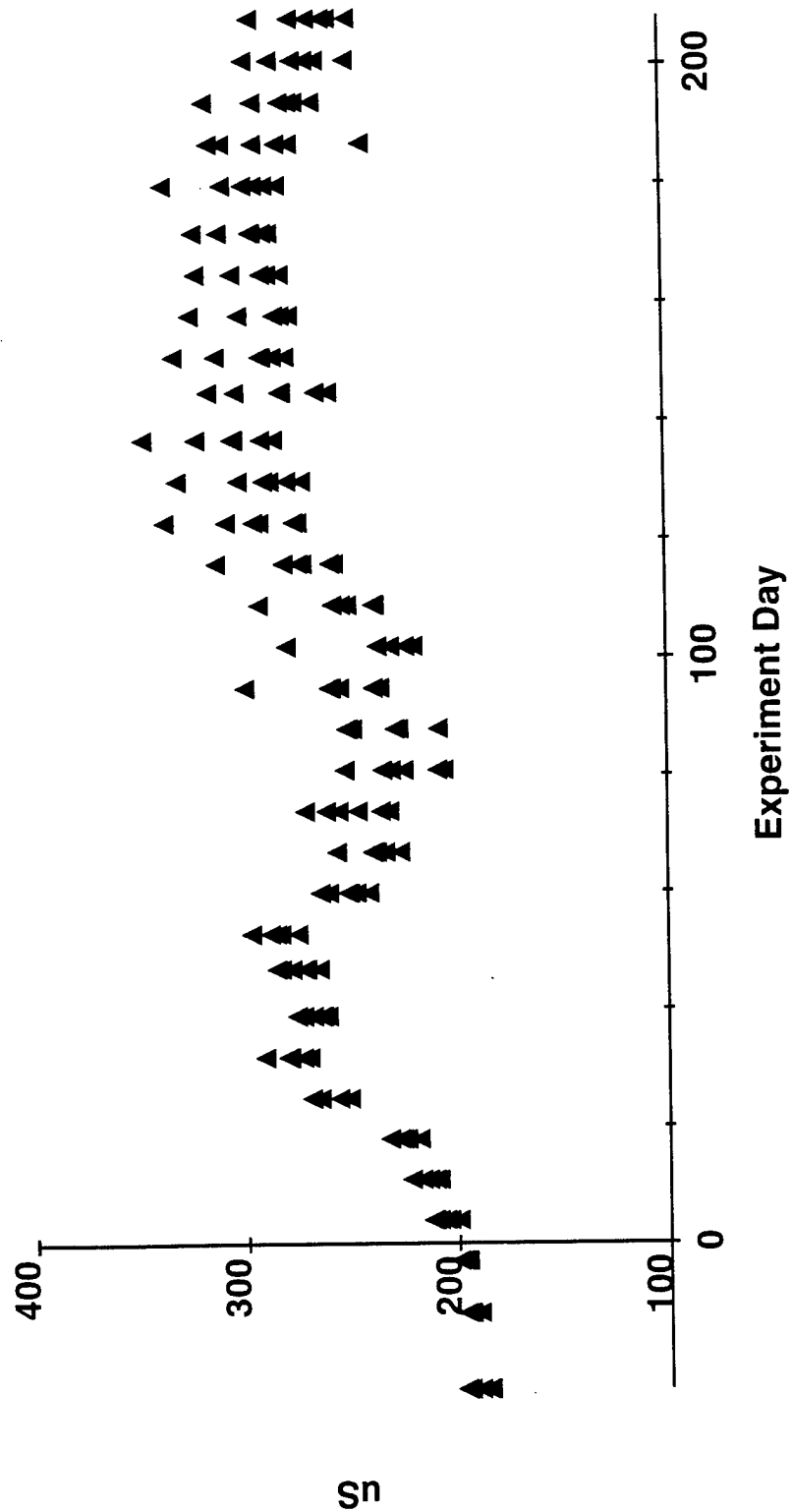
Figure 86a. Water Quality
Dissolved Oxygen, amDO1 - High Dose
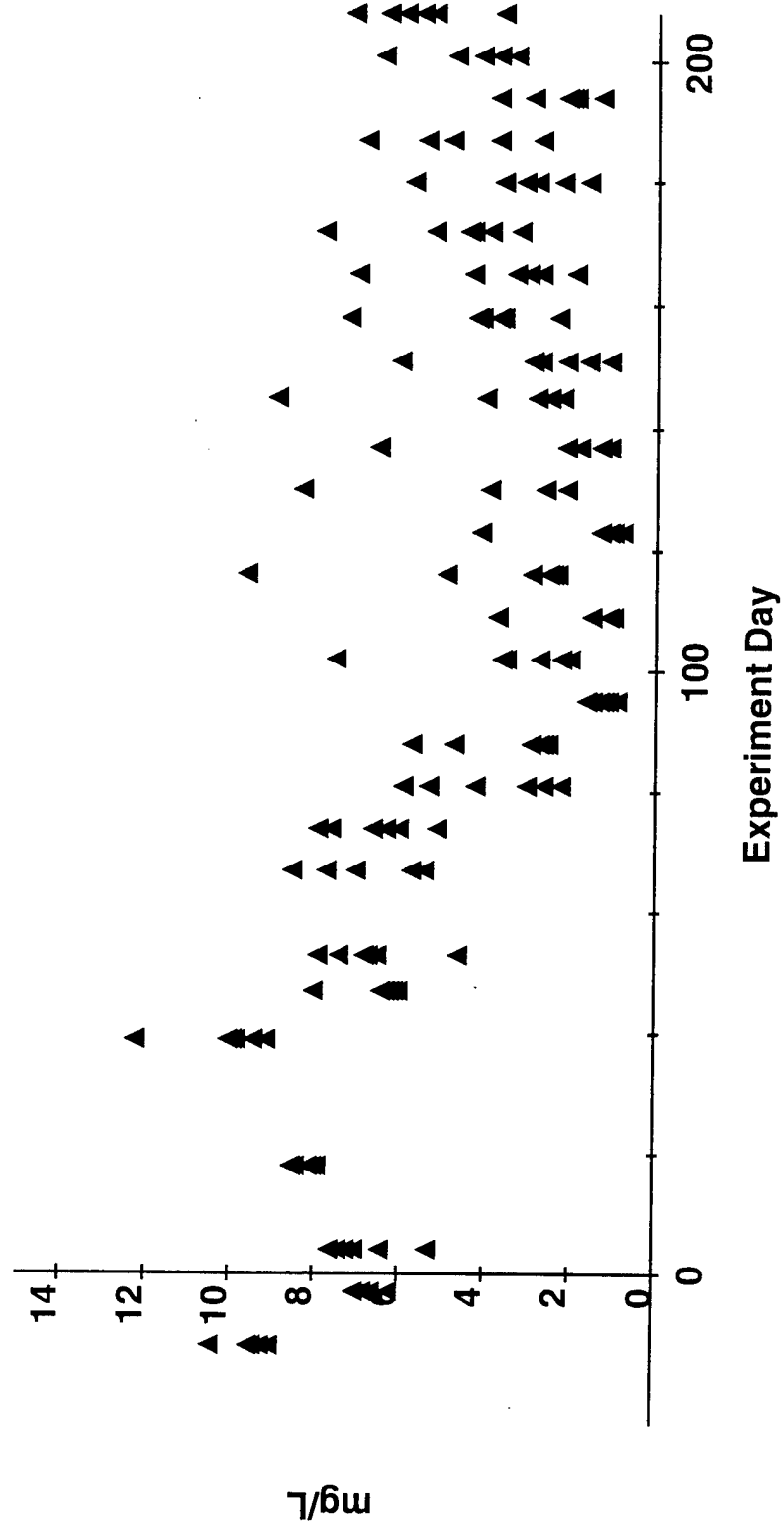
Figure 86b. Water Quality
Dissolved Oxygen, amDO1 - Mid-dose
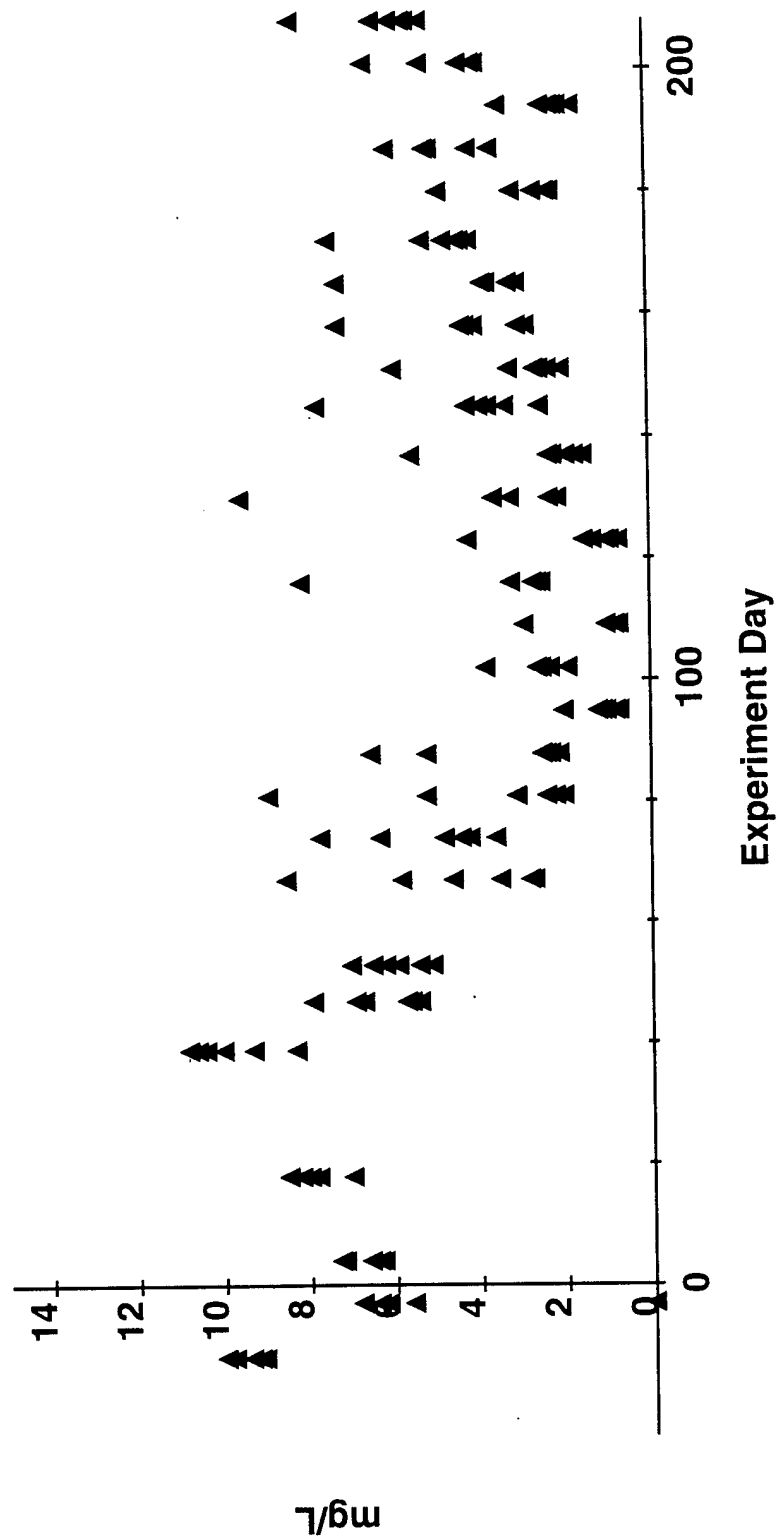
Figure 86c. Water Quality
Dissolved Oxygen, amDO1 - Low Dose
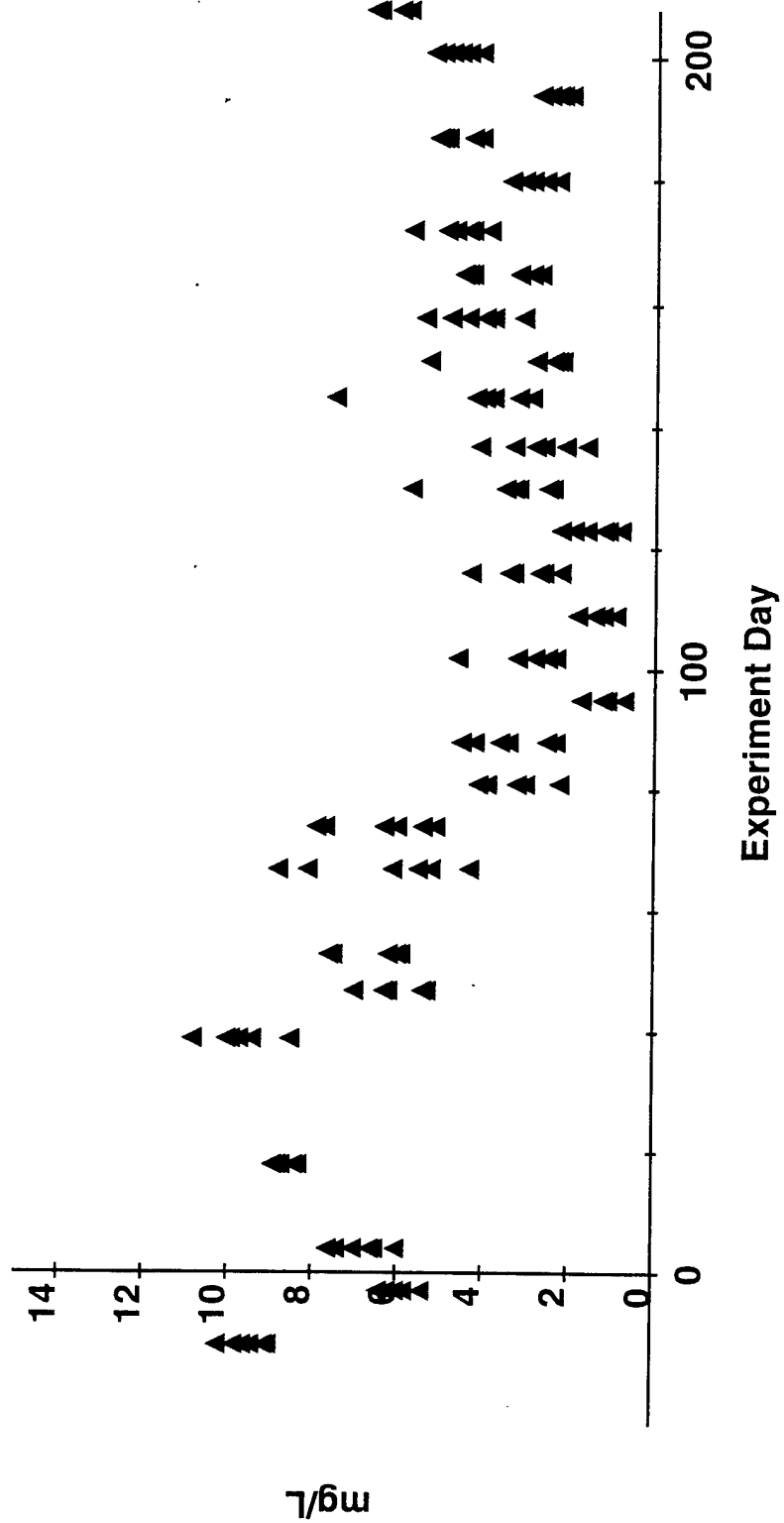
Figure 86d. Water Quality
Dissolved Oxygen, amDO1 - Undosed
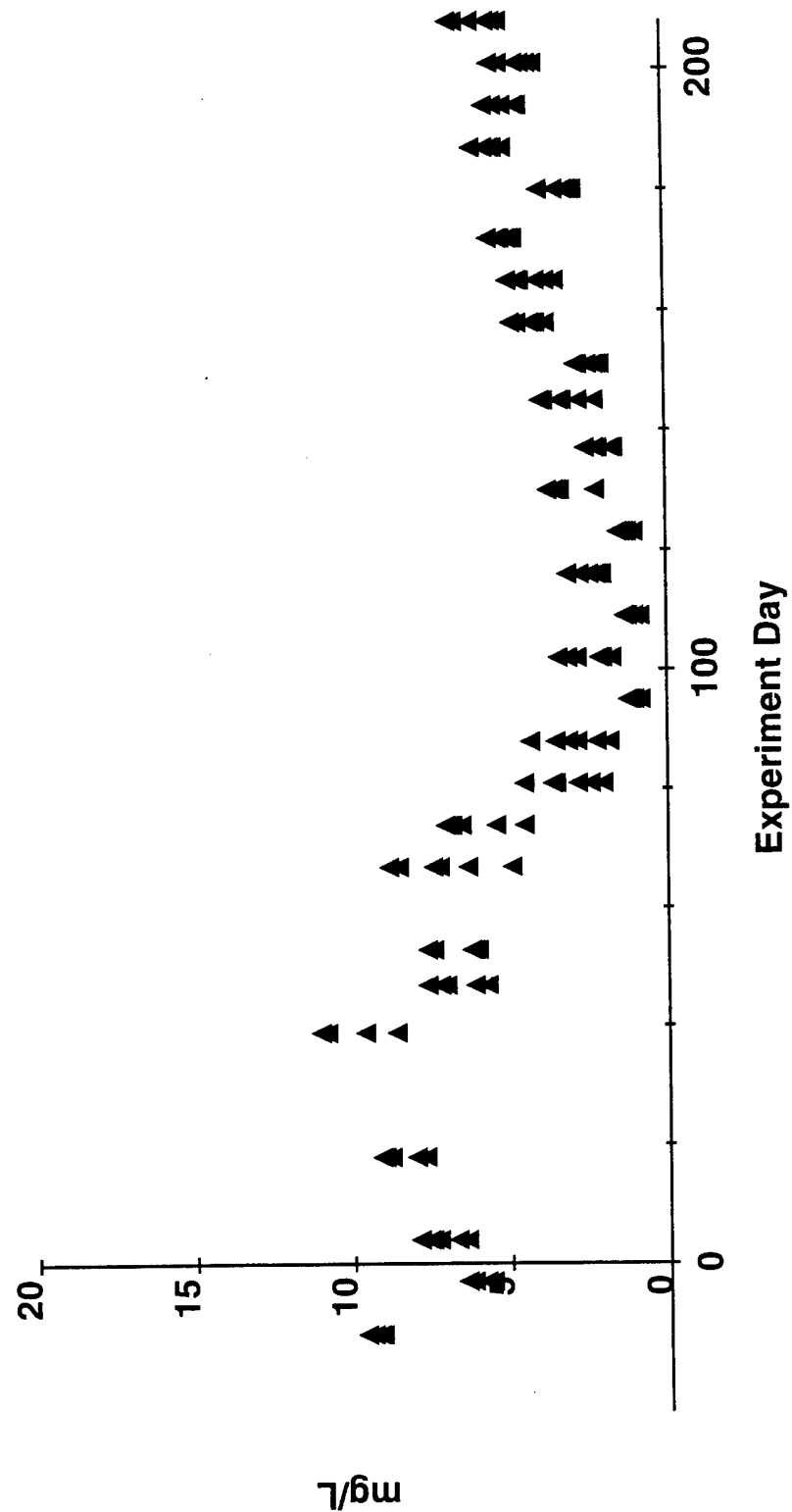
Figure 87a. Water Quality
Dissolved Oxygen, pmDO2 - High Dose
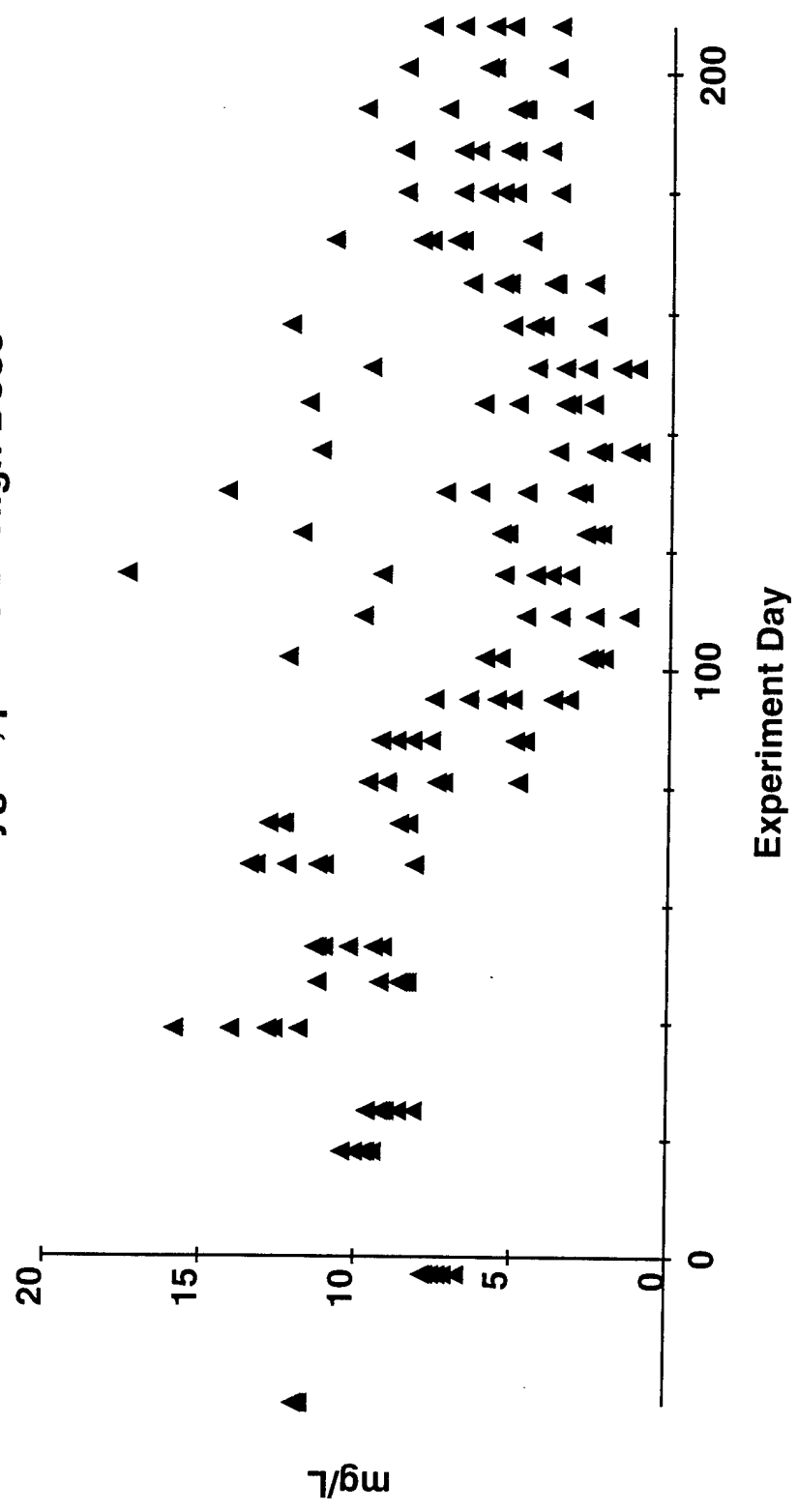
Figure 87b. Water Quality
Dissolved Oxygen, pmDO2 - Mid-dose
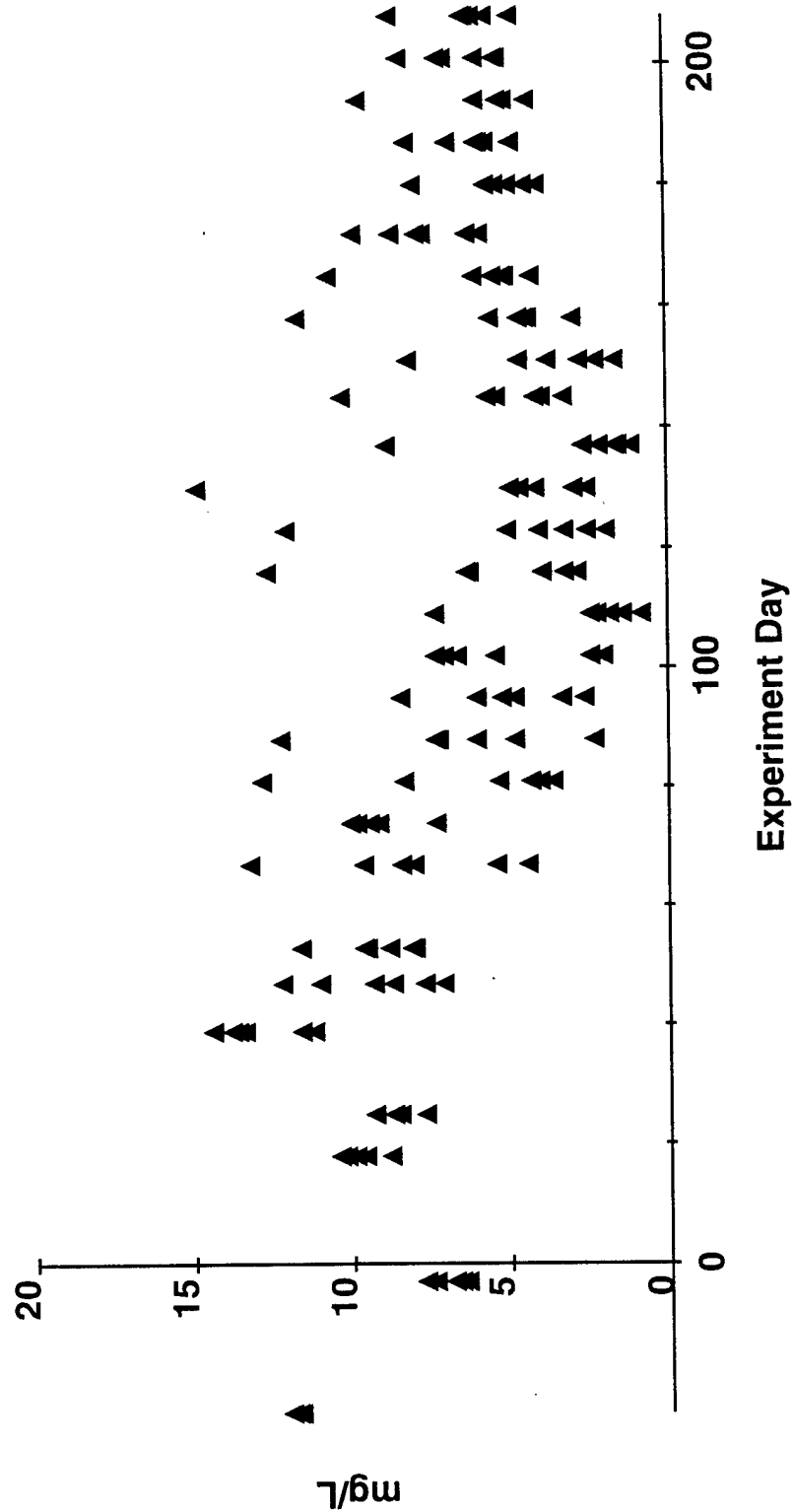
Figure 87c. Water Quality
Dissolved Oxygen, pmDO2 - Low Dose
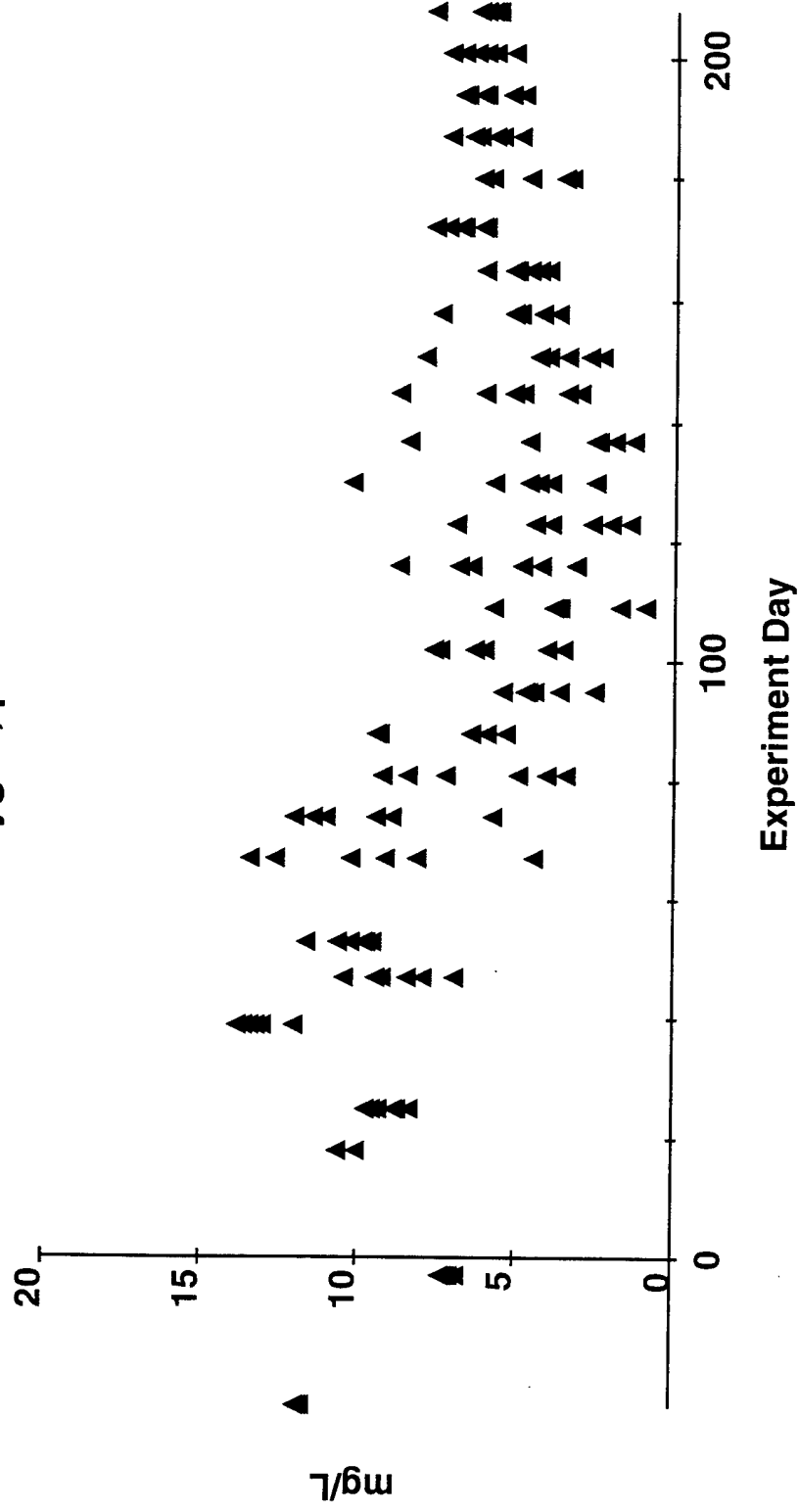
Figure 87d. Water Quality
Dissolved Oxygen, pmDO2 - Undosed
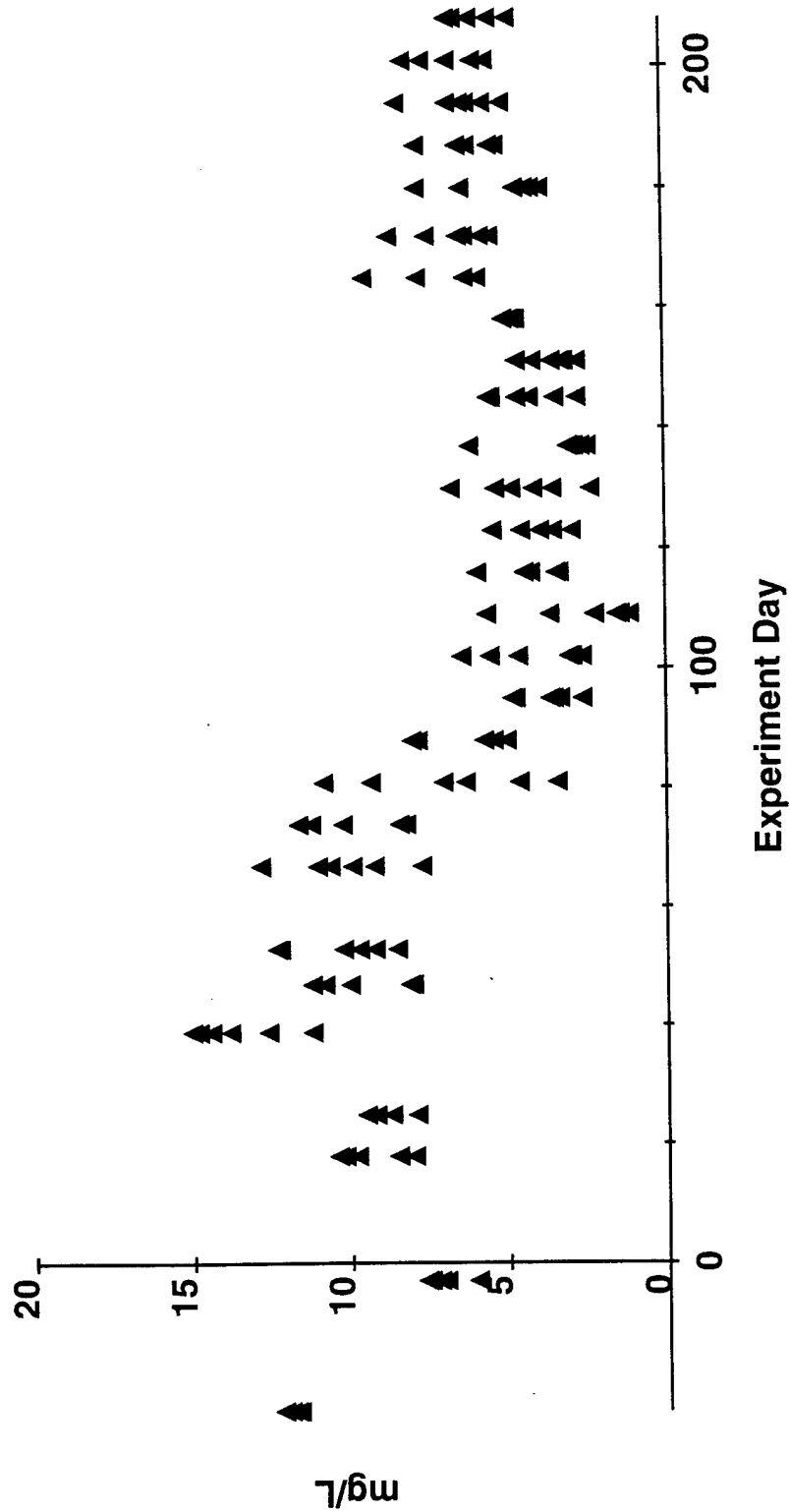
Figure 88a. Water Quality
Dissolved Oxygen, amDO3 - High Dose
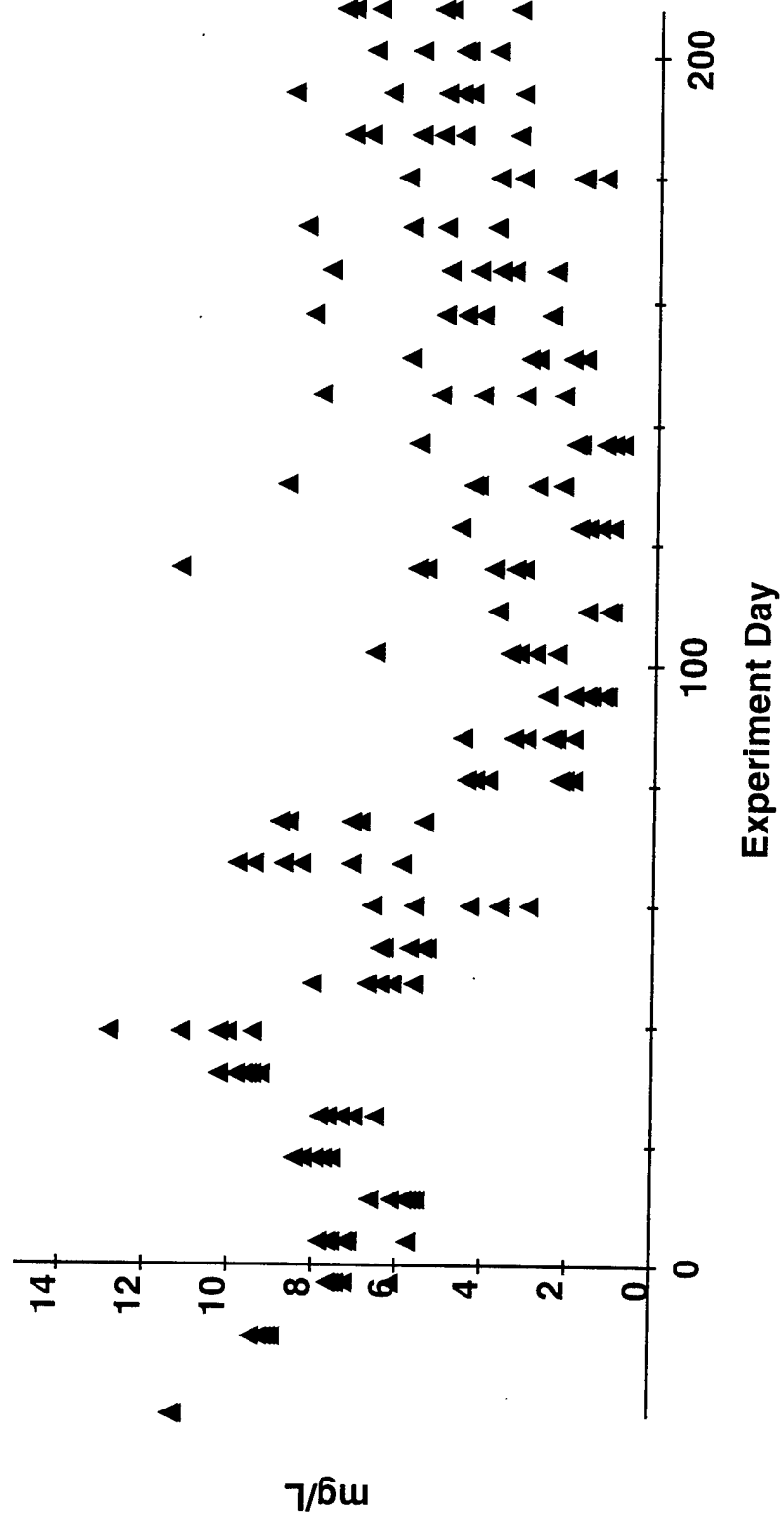
Figure 88b. Water Quality
Dissolved Oxygen, amDO3 - Mid-dose
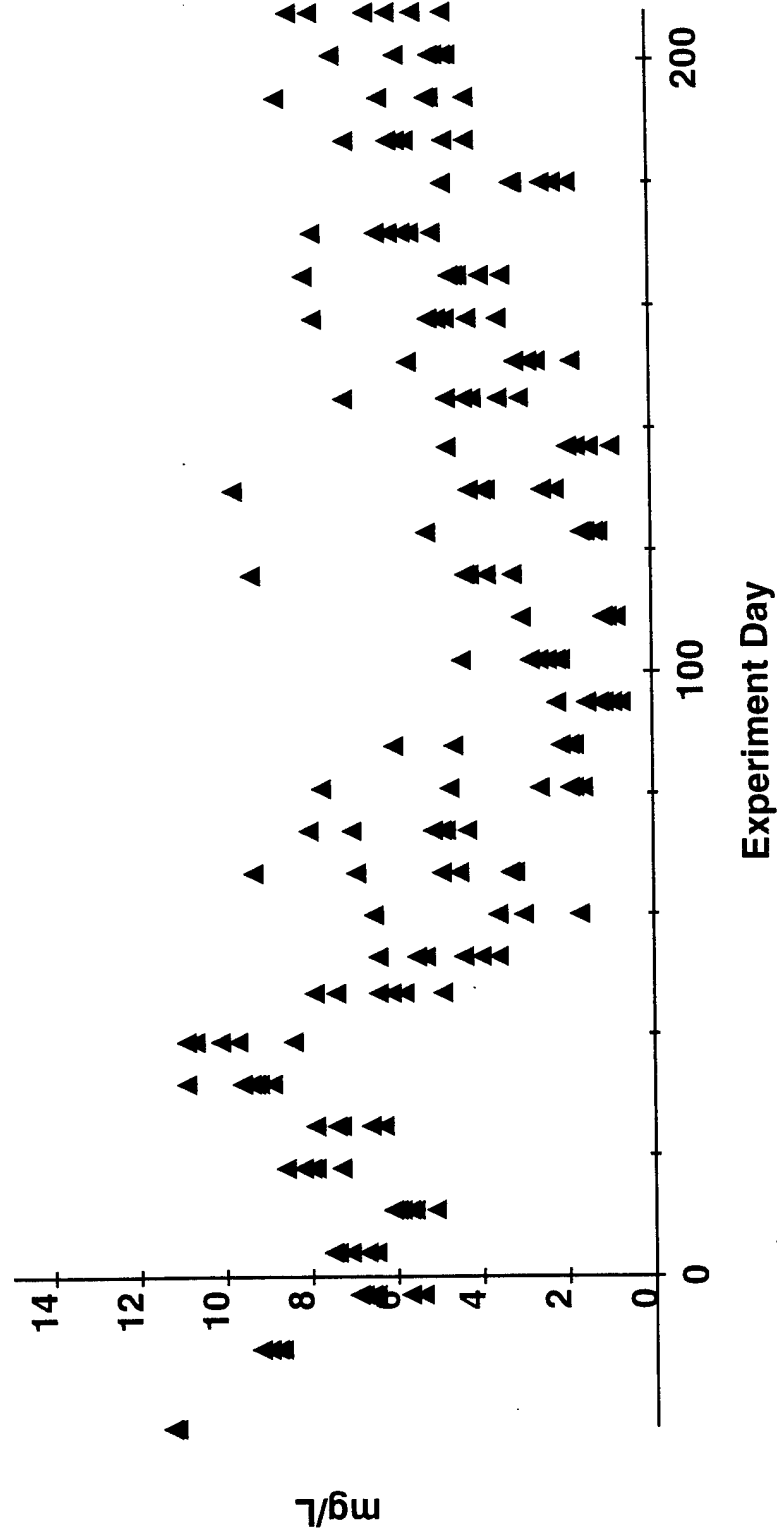
Figure 88c. Water Quality
Dissolved Oxygen, amDO3 - Low Dose
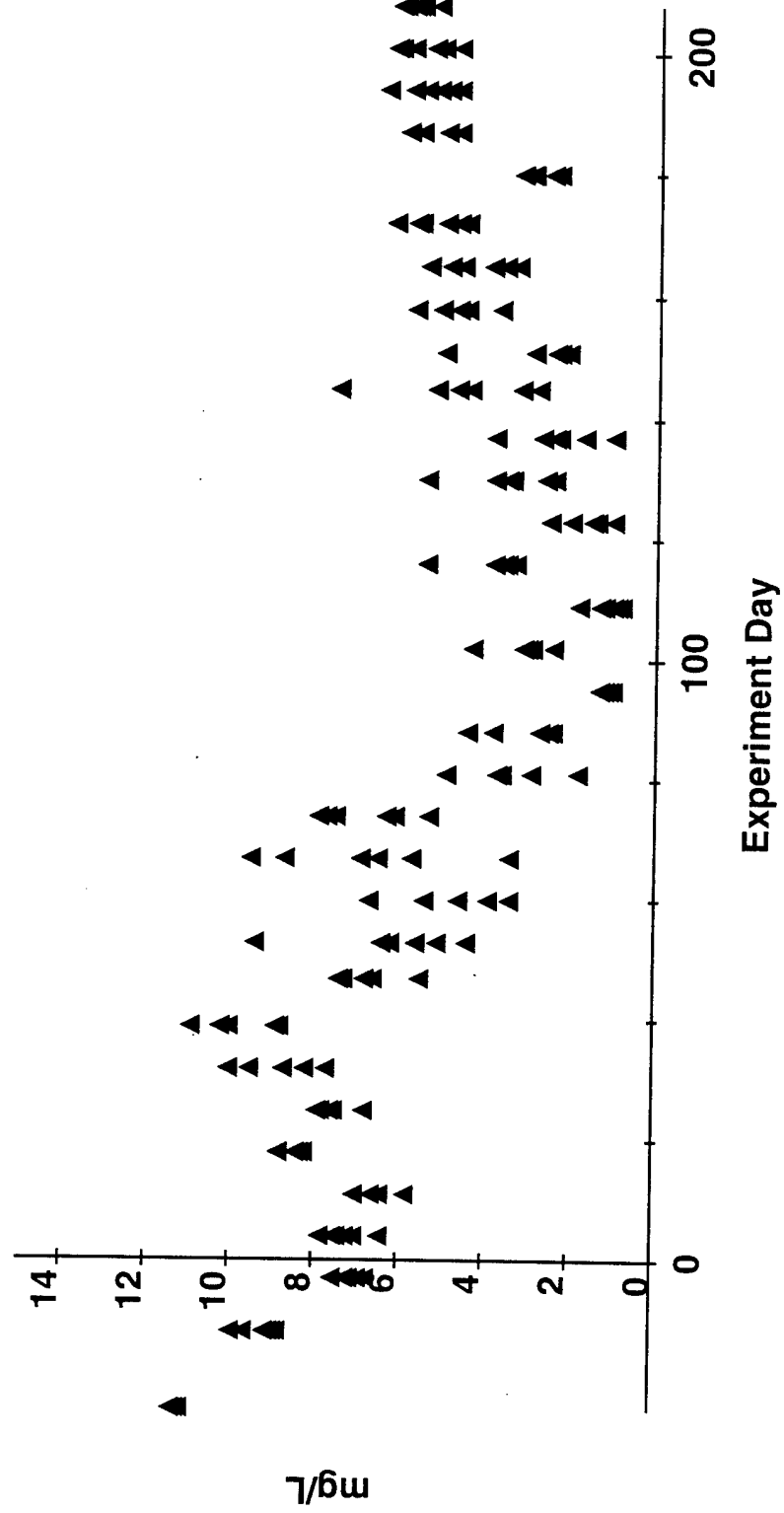
Figure 88d. Water Quality
Dissolved Oxygen, amDO3 - Undosed
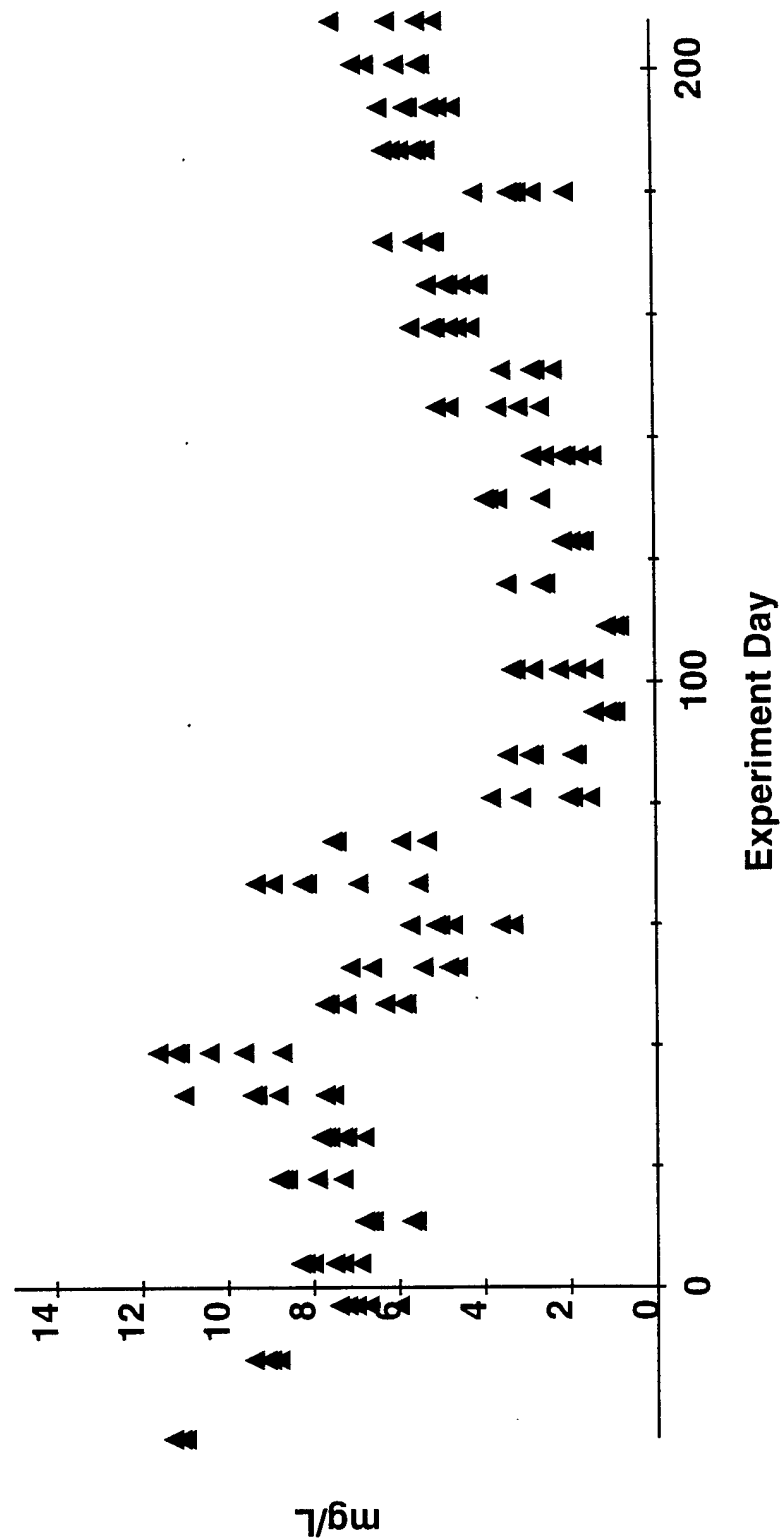
Figure 89a. Water Quality
Hardness - High Dose
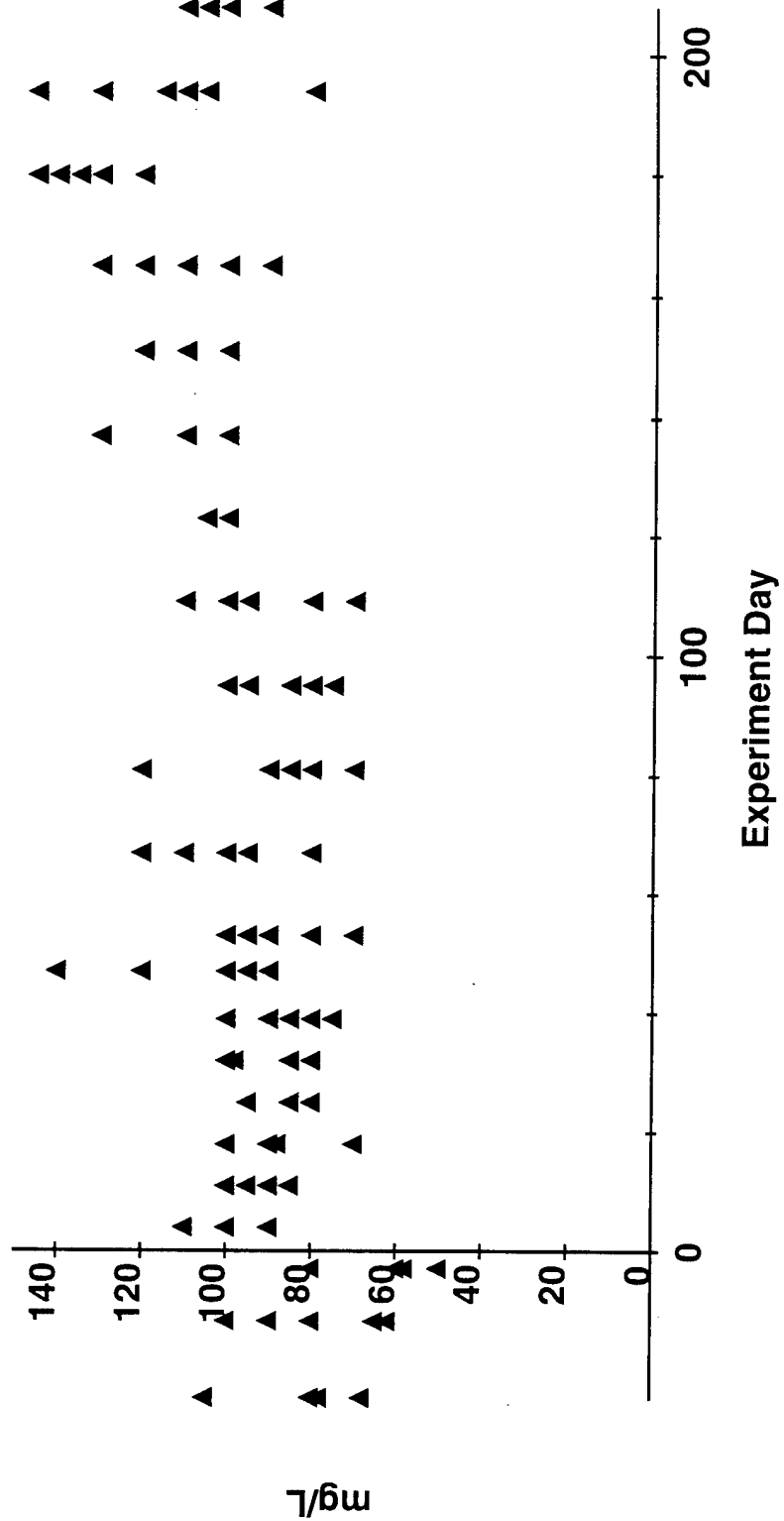
Figure 89b. Water Quality
Hardness - Mid-dose
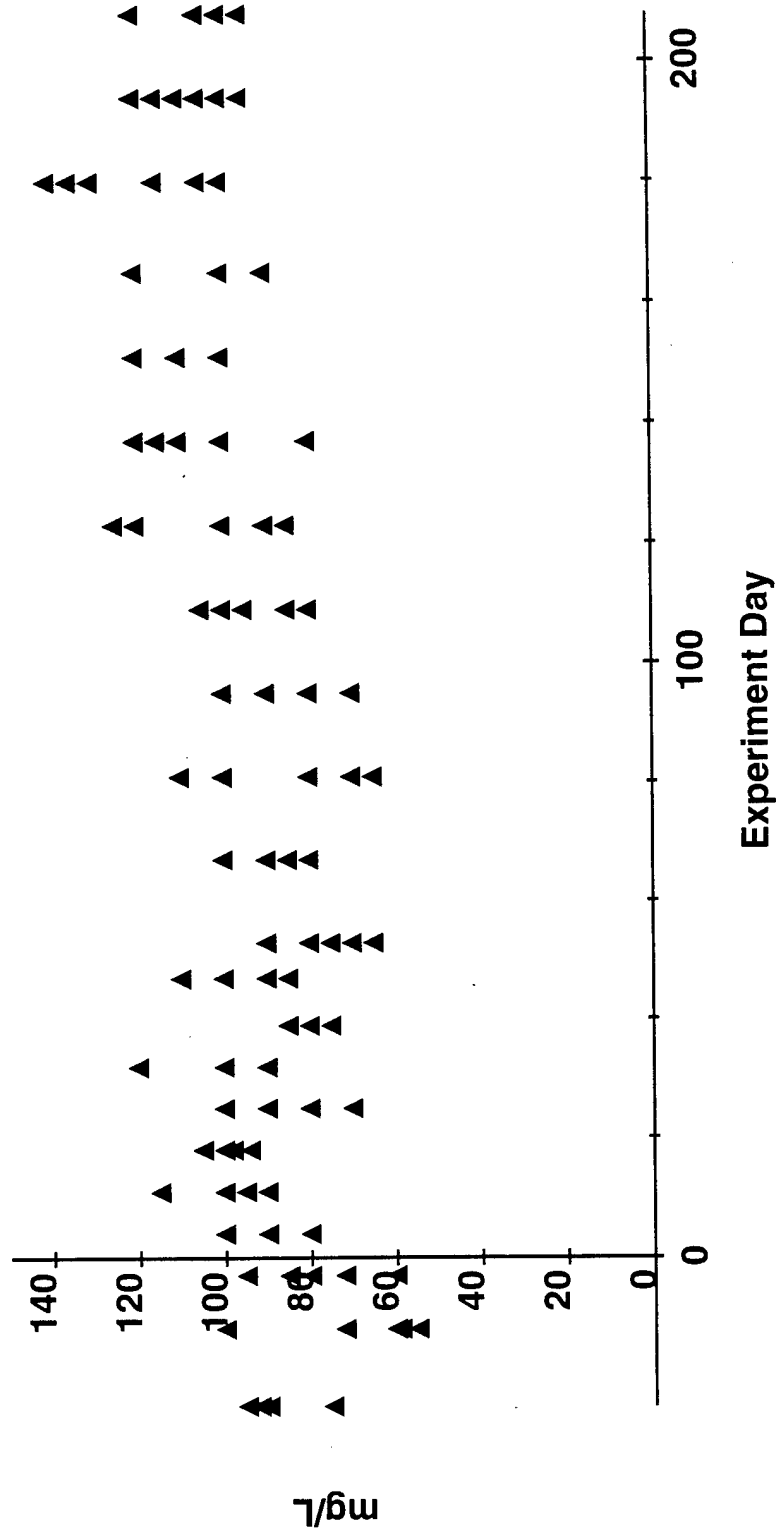
Figure 89c. Water Quality
Hardness - Low Dose
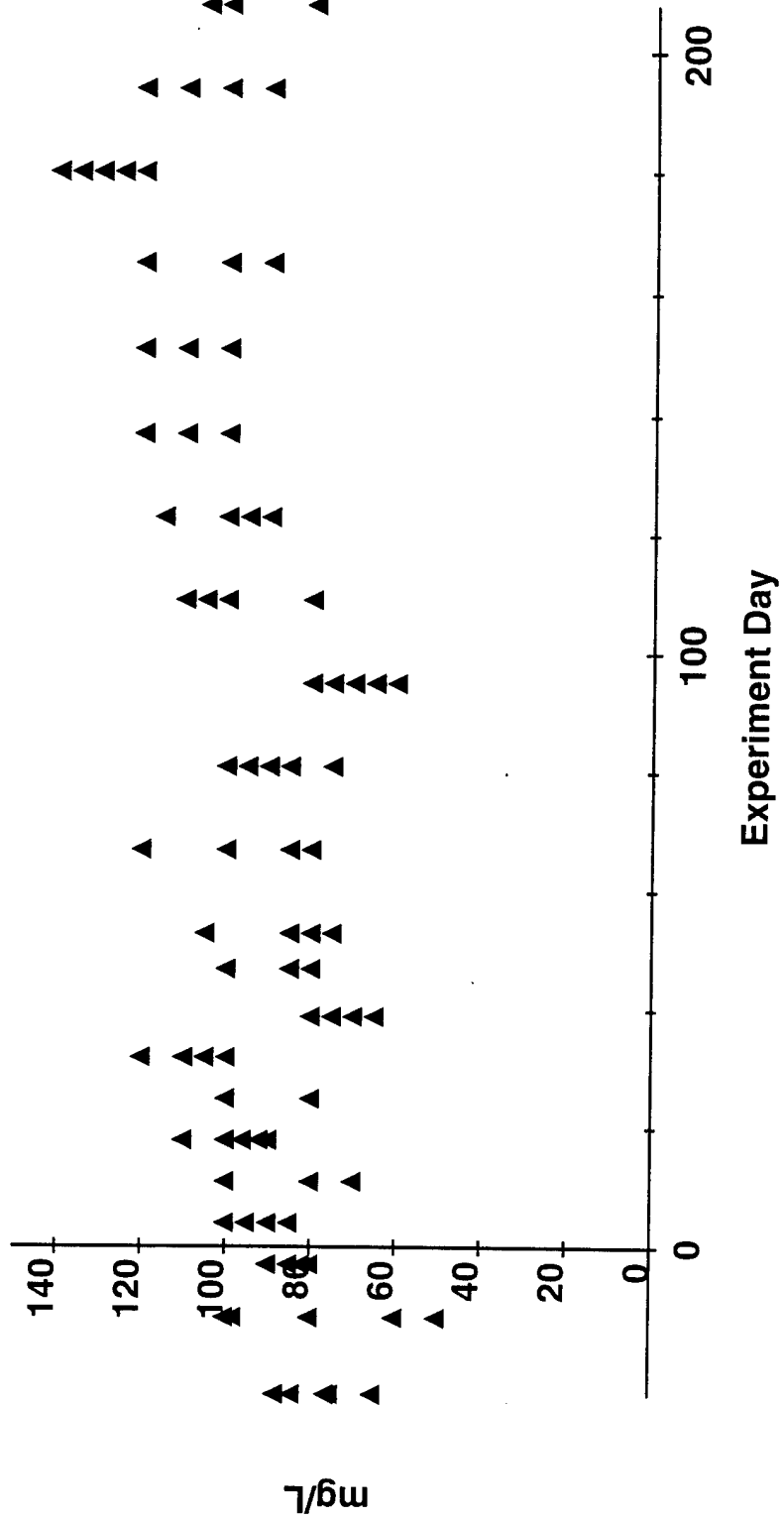
Figure 89d. Water Quality
Hardness - Undosed
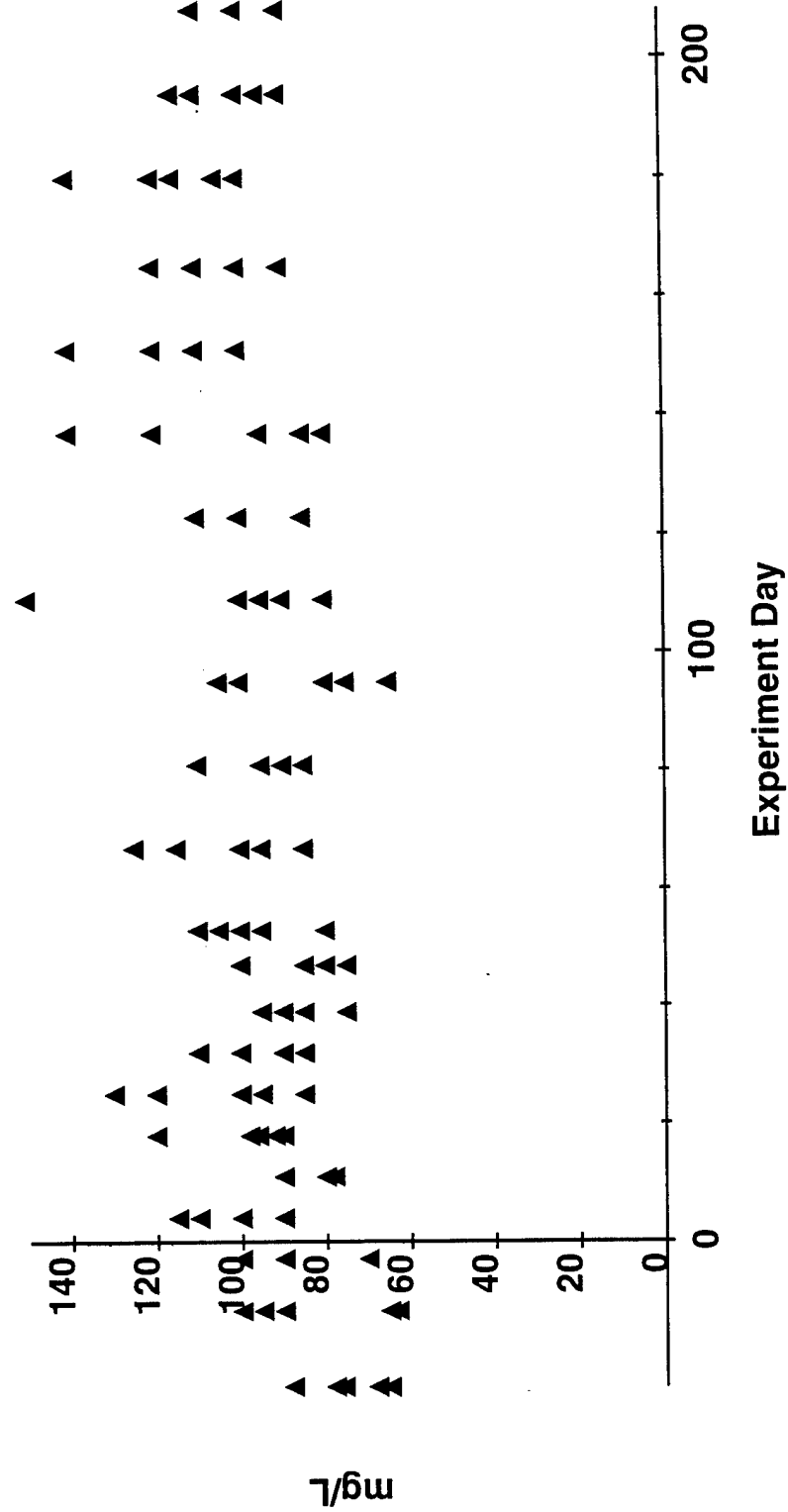
Figure 90a. Water Quality
Nitrate/Nitrite - High Dose
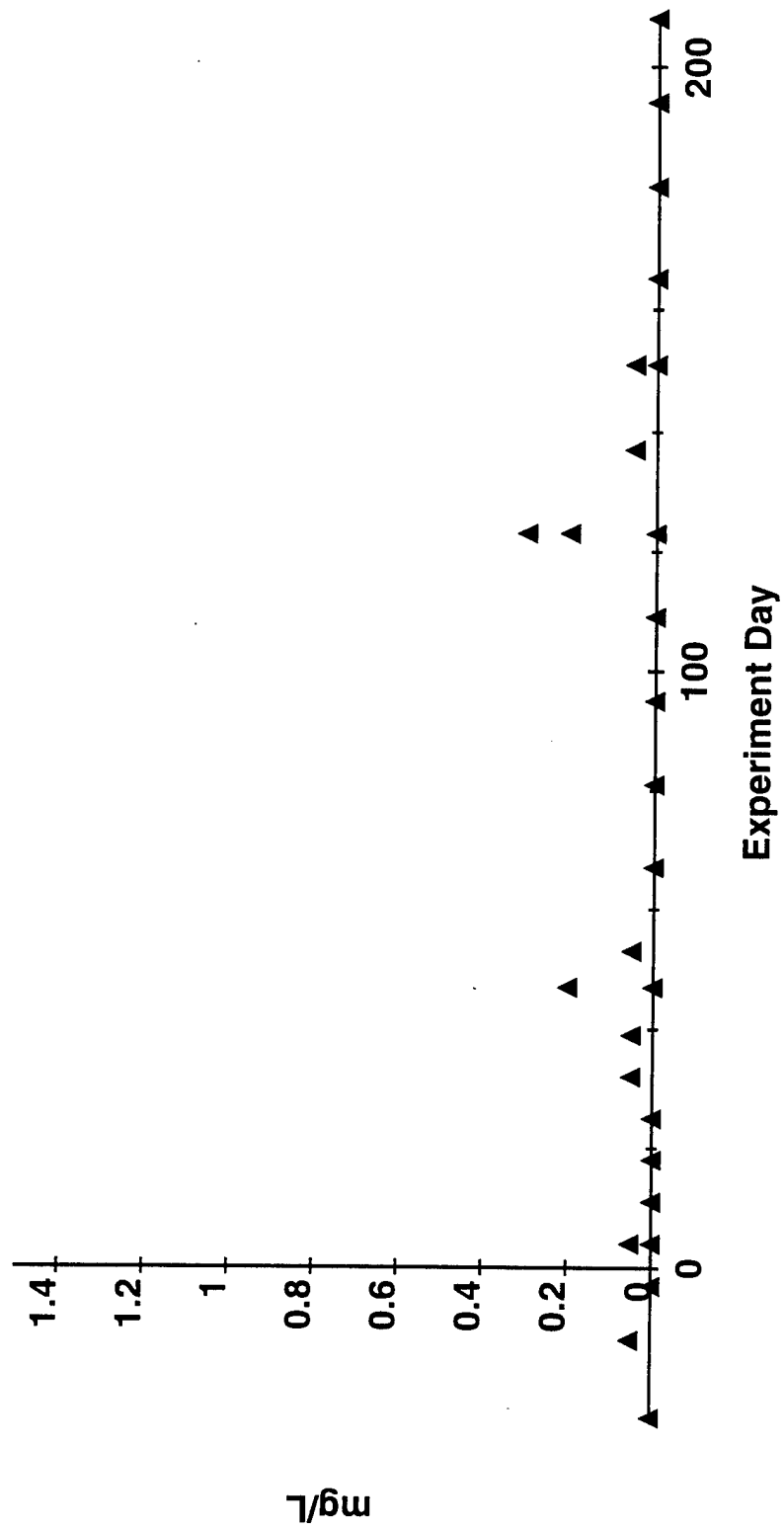
Figure 90b. Water Quality
Nitrate/Nitrite - Mid-dose
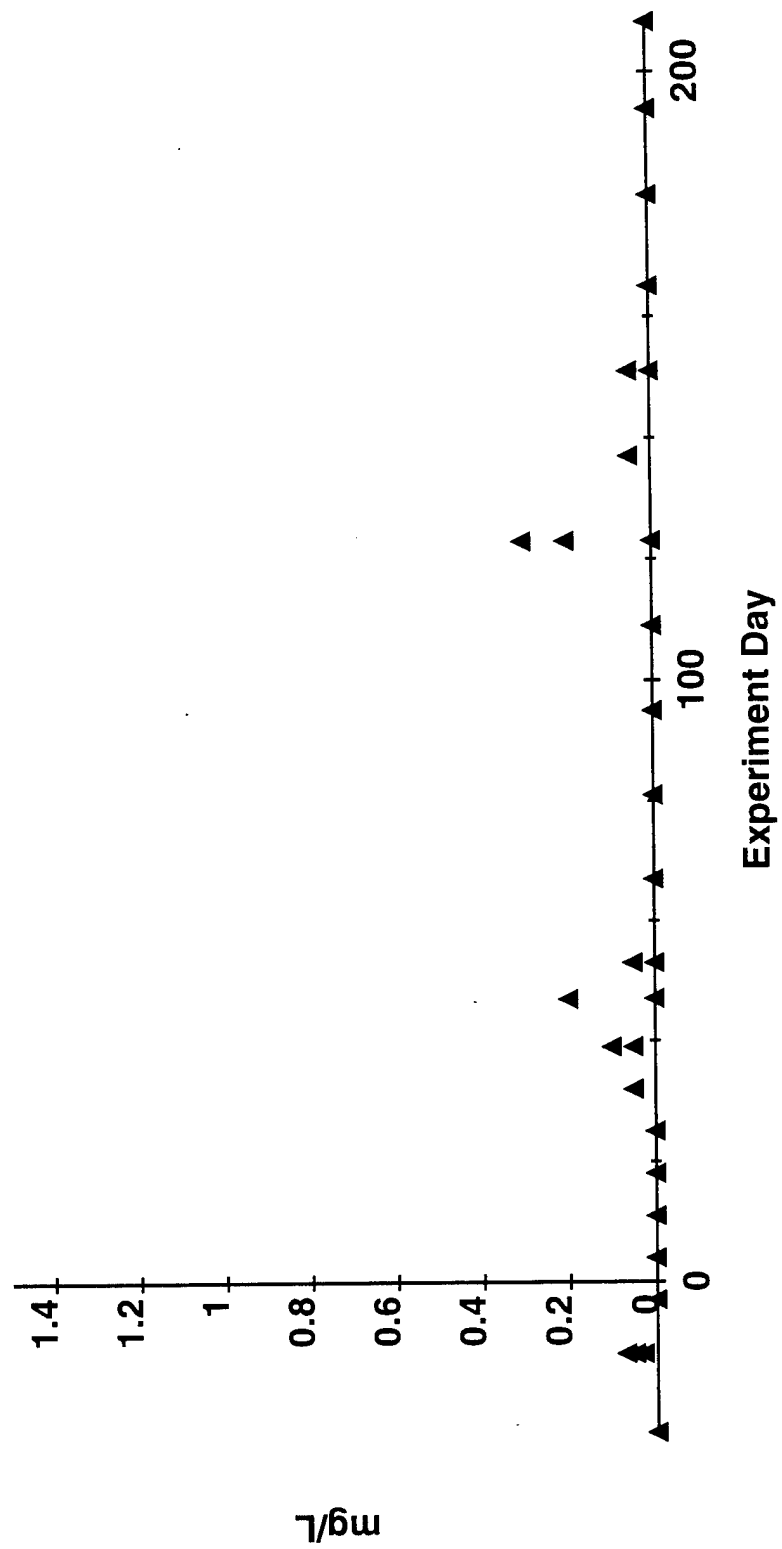
Figure 90c. Water Quality
Nitrate/Nitrite - Low Dose
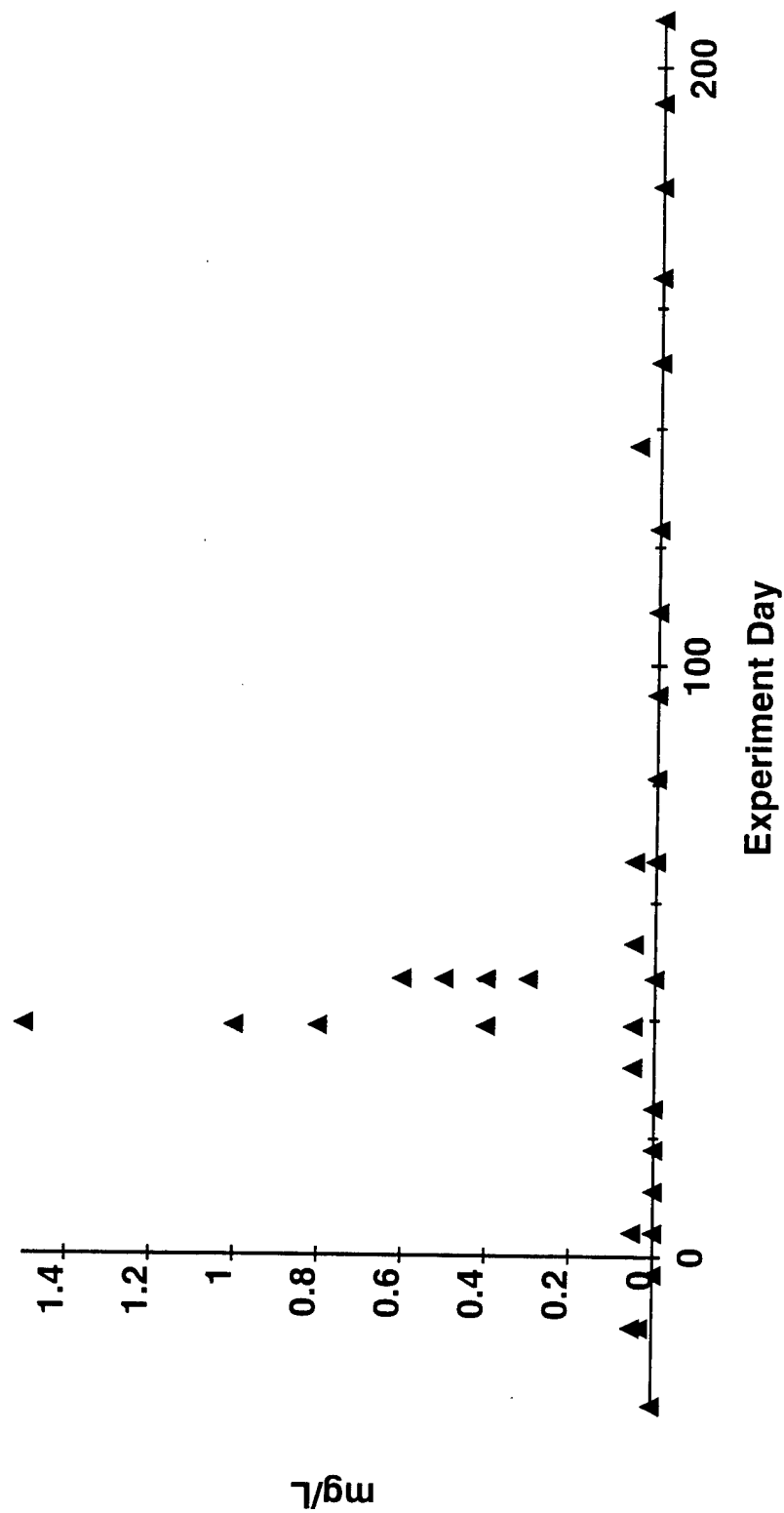
Figure 90d. Water Quality
Nitrate/Nitrite - Undosed

Experiment Day

mg/L
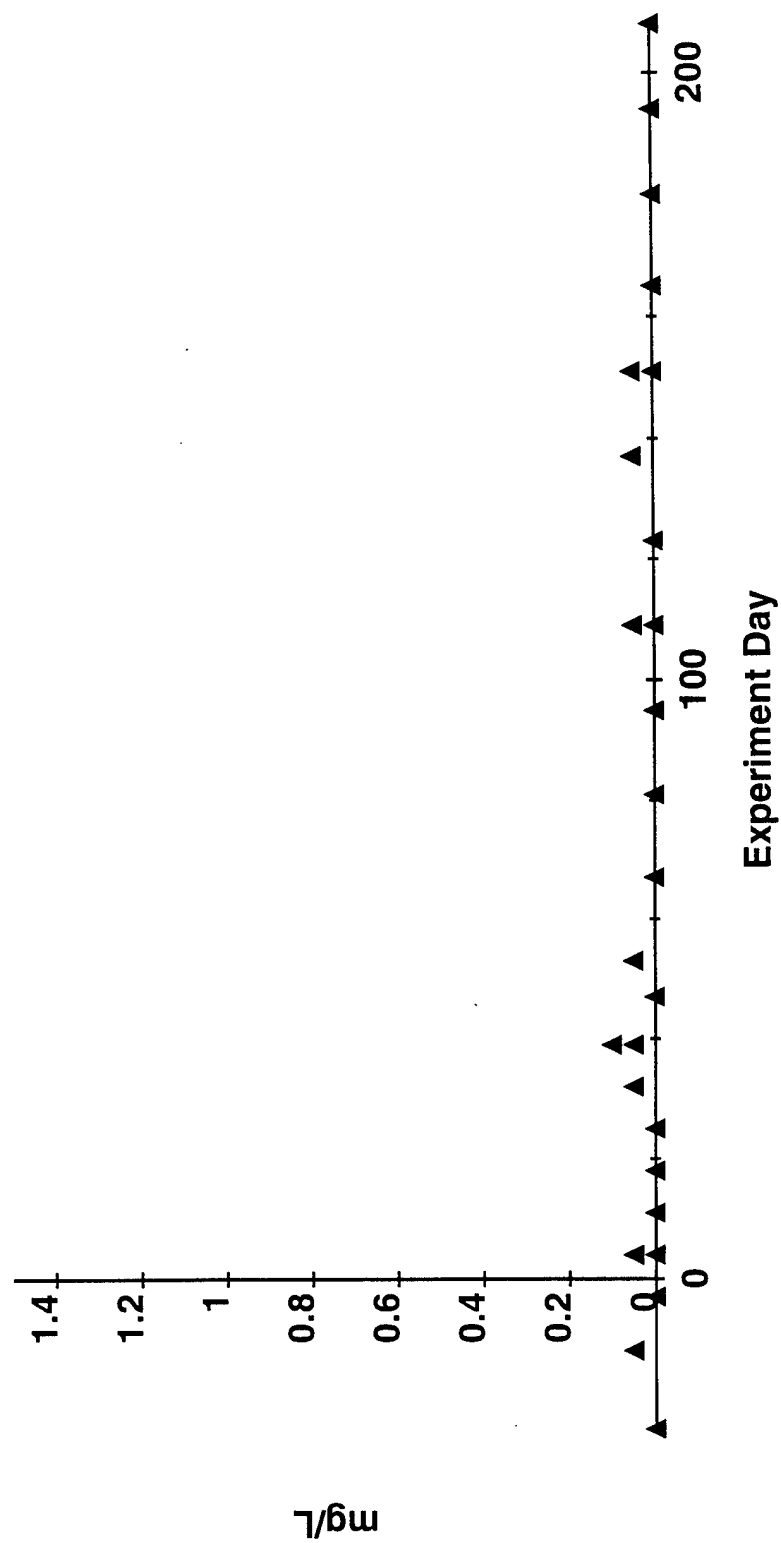
Figure 91a. Water Quality
pH - High Dose
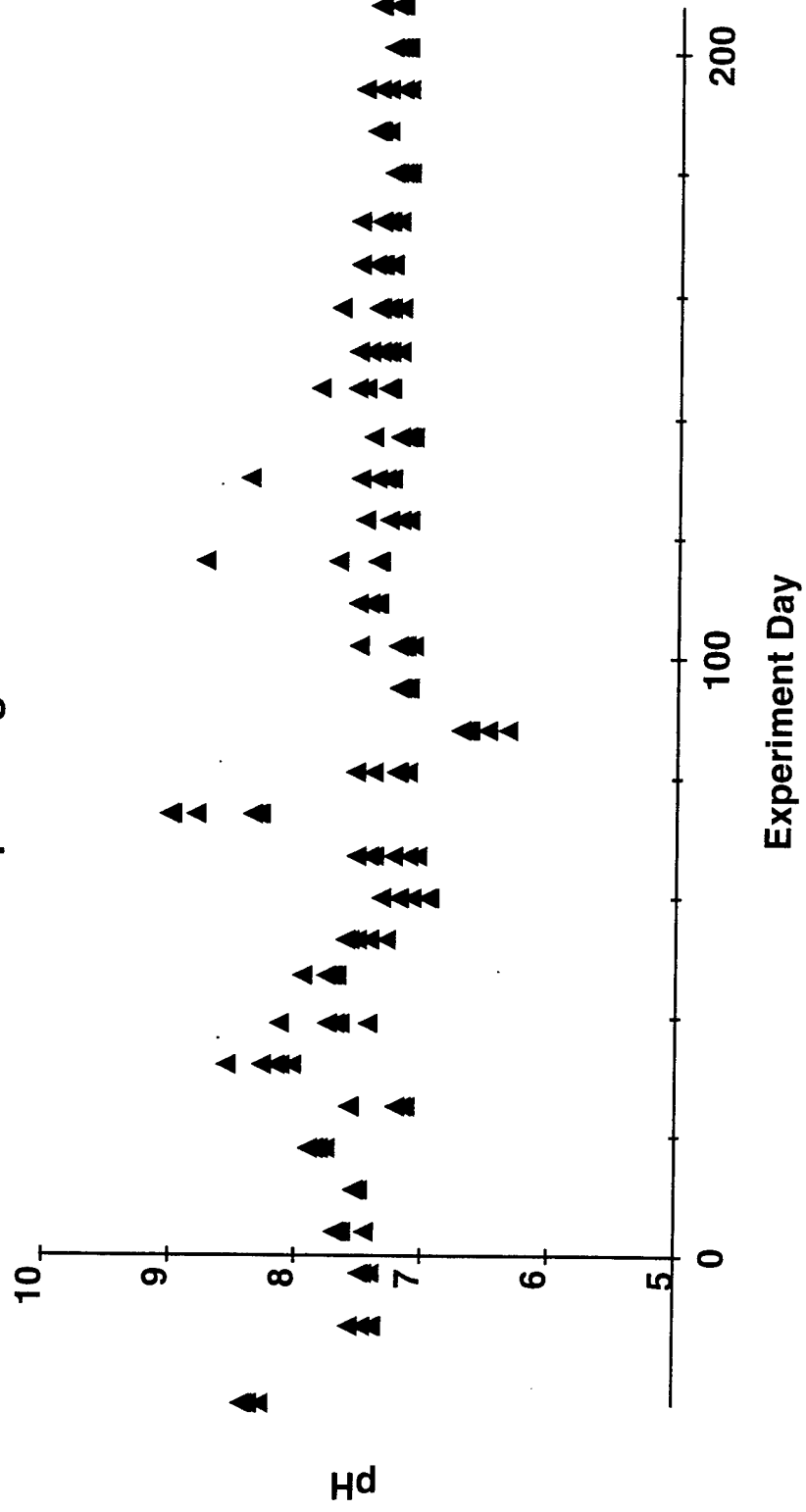
Figure 91b. Water Quality
pH - Mid-dose
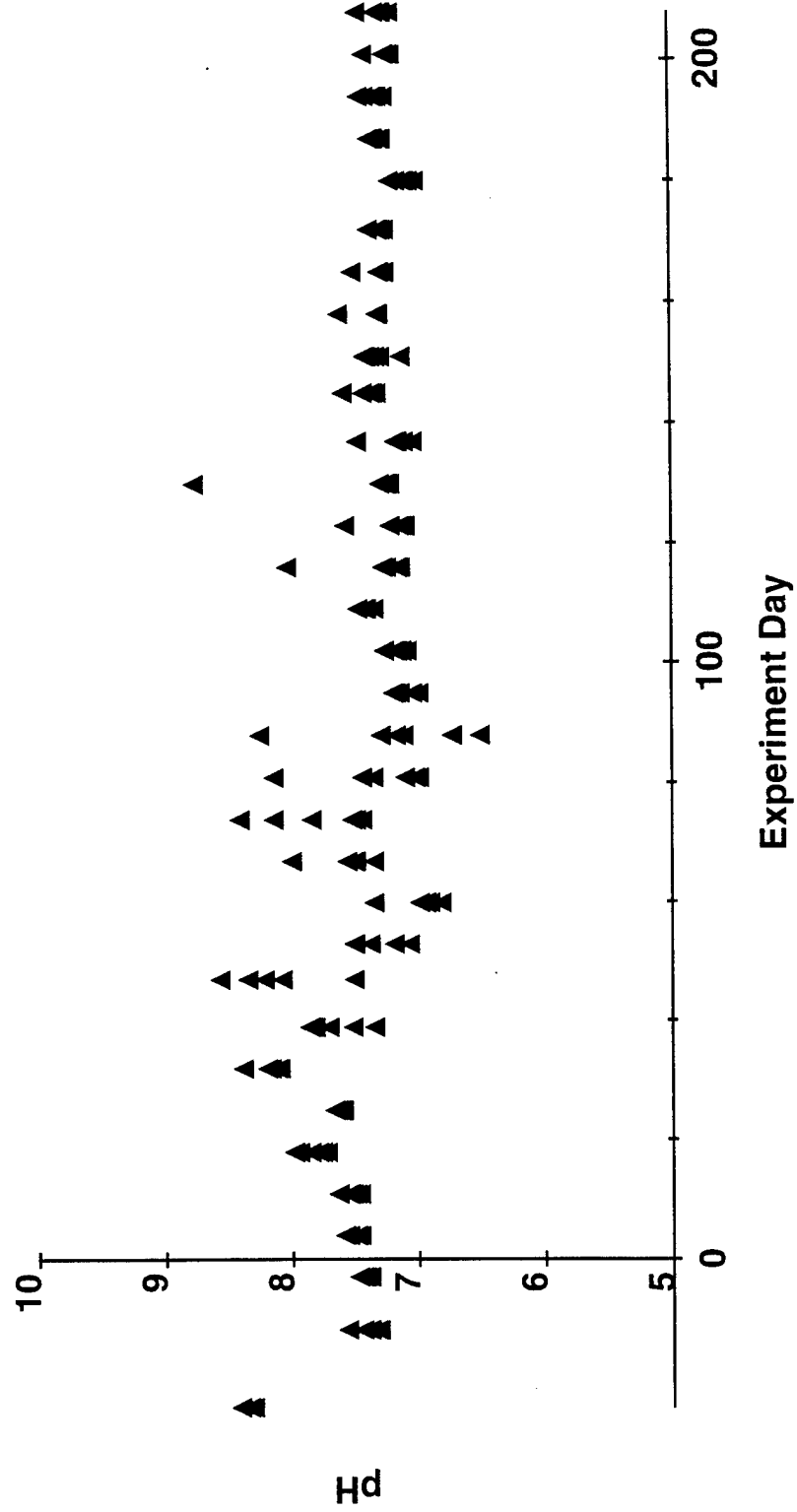
Figure 91c. Water Quality
pH - Low Dose

pH

Experiment Day
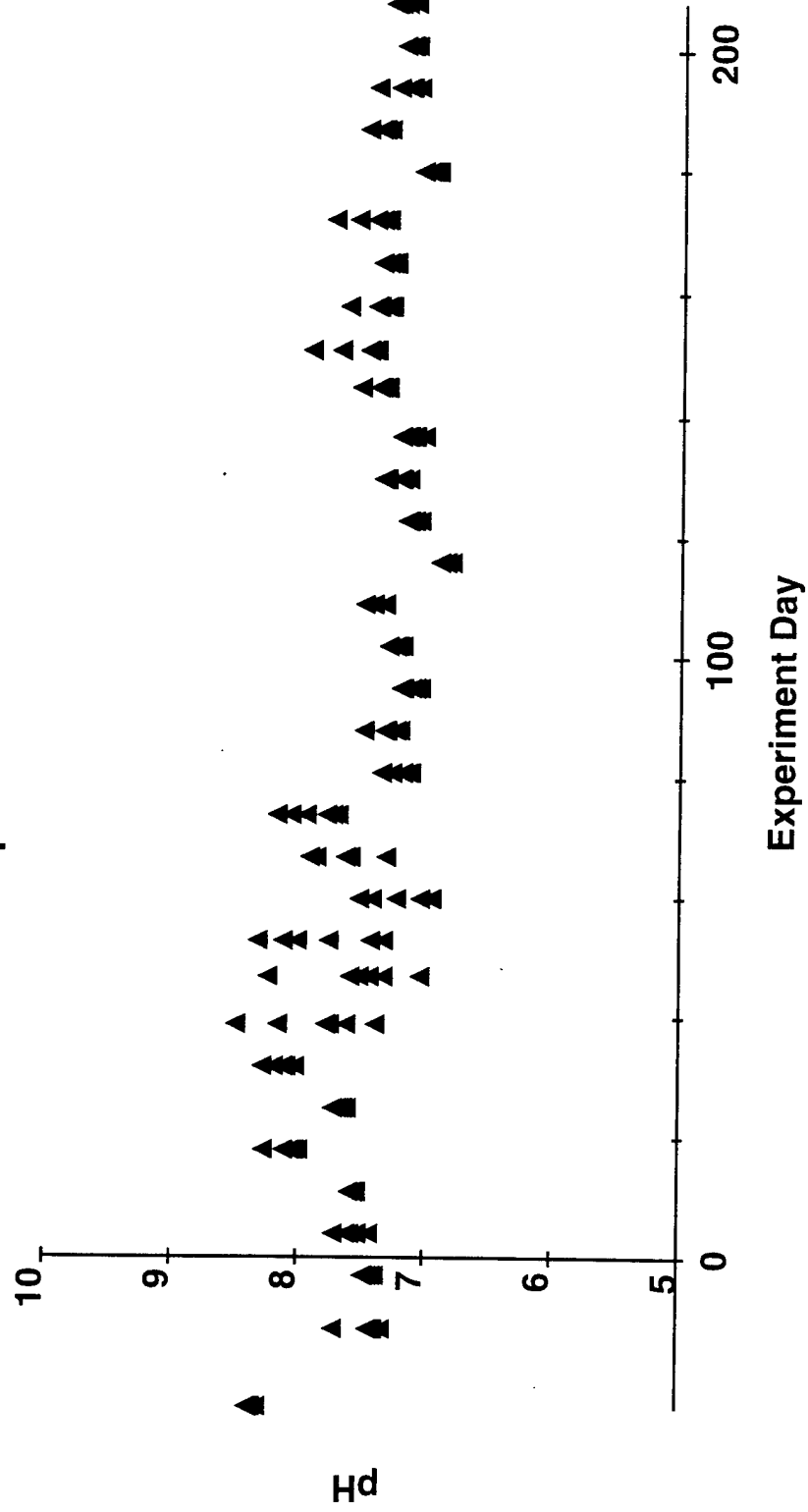
Figure 91d. Water Quality
pH - Undosed
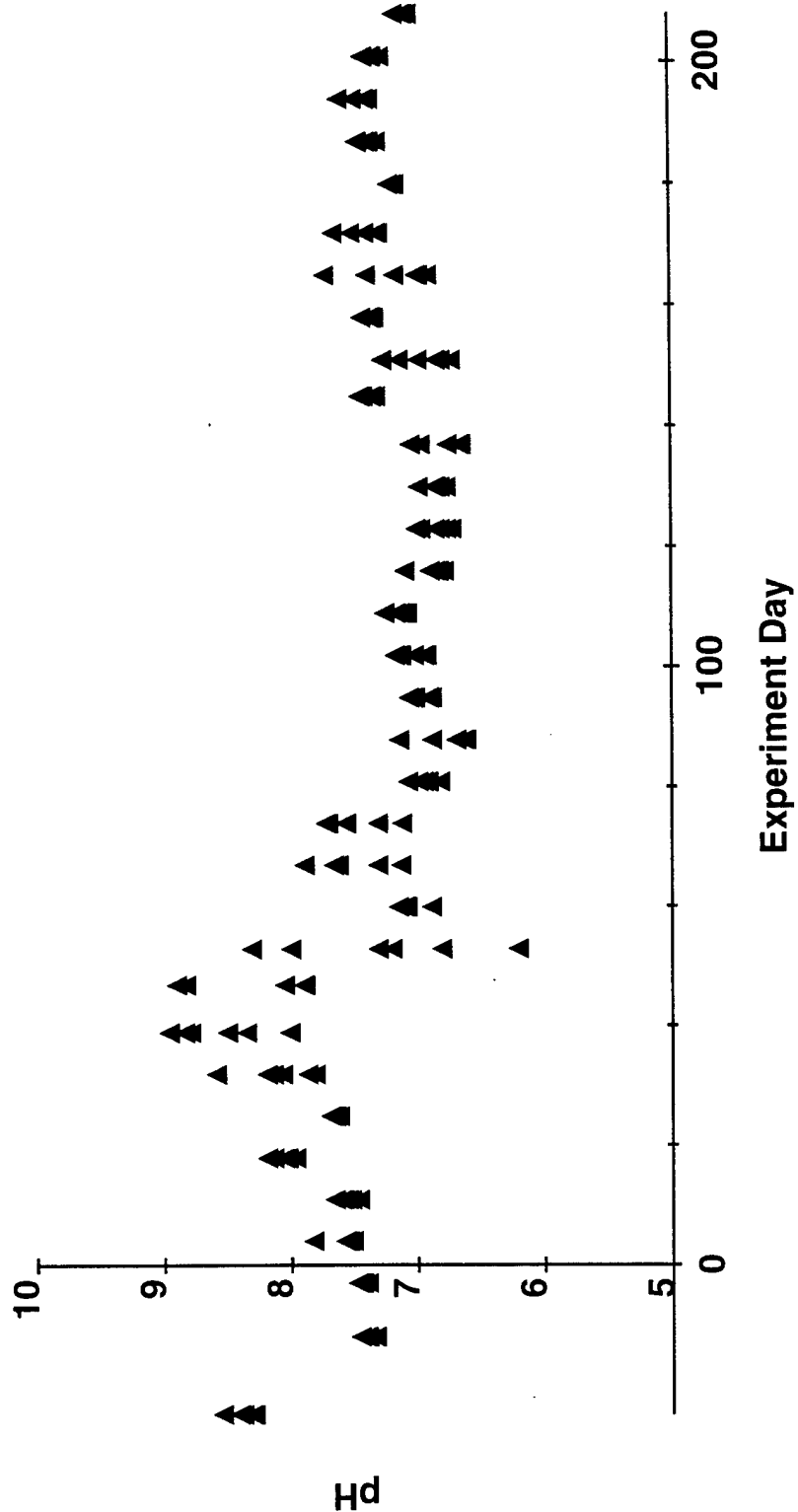
Figure 92a. Water Quality
Soluble Reactive Phosphate - High Dose
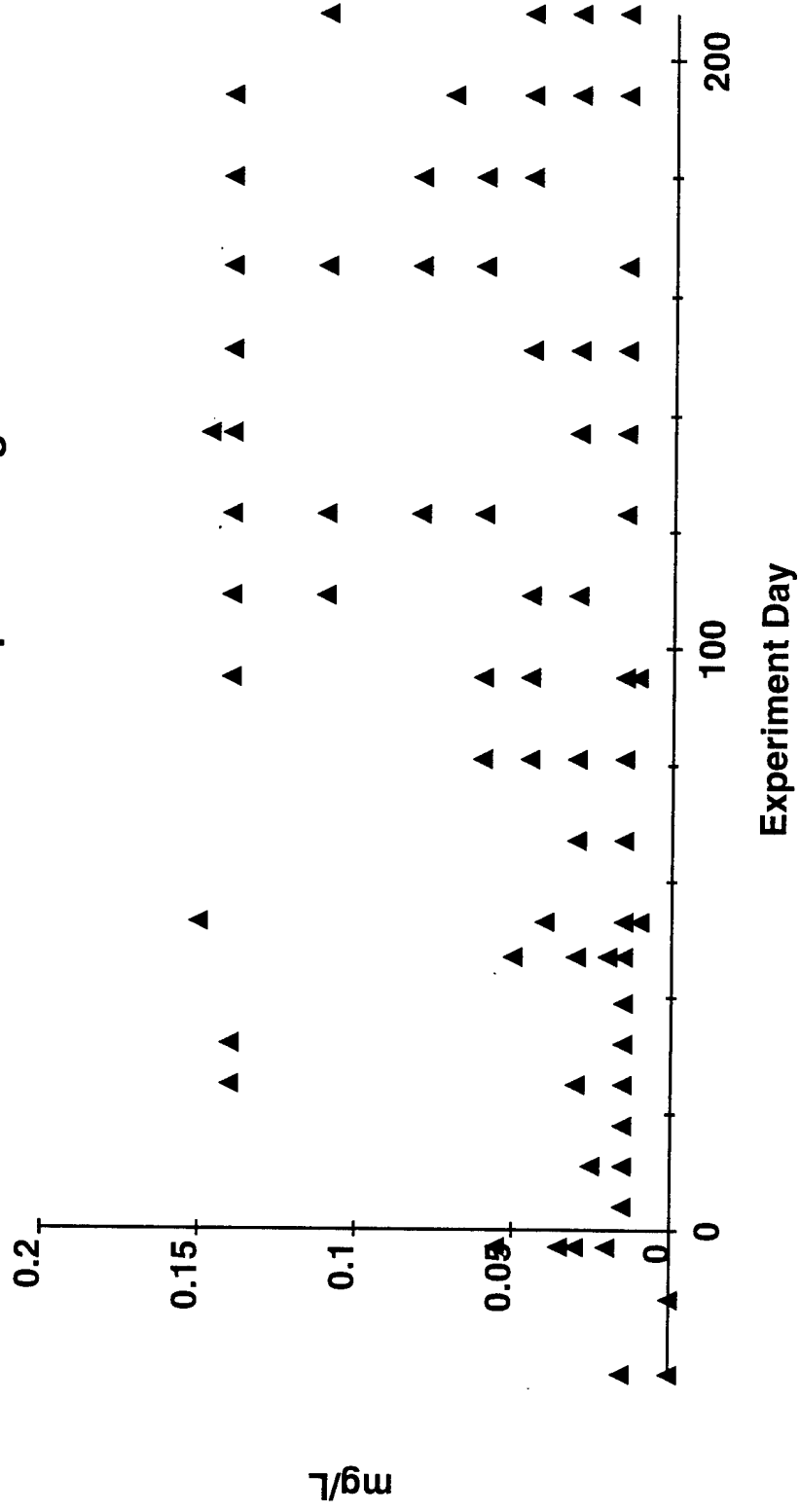
Figure 92b. Water Quality
Soluble Reactive Phosphate - Mid-dose
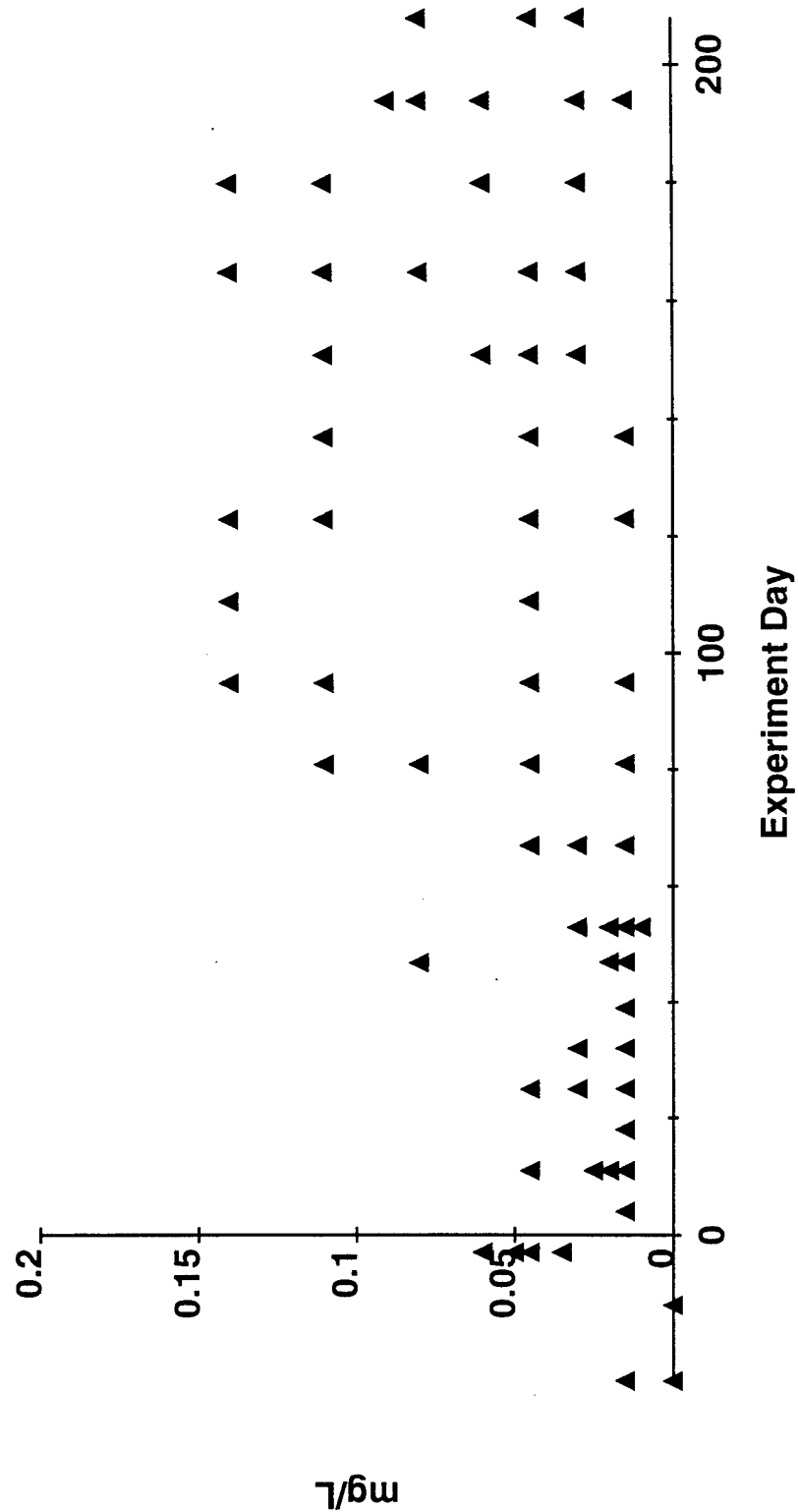
Figure 92c. Water Quality
Soluble Reactive Phosphate - Low Dose
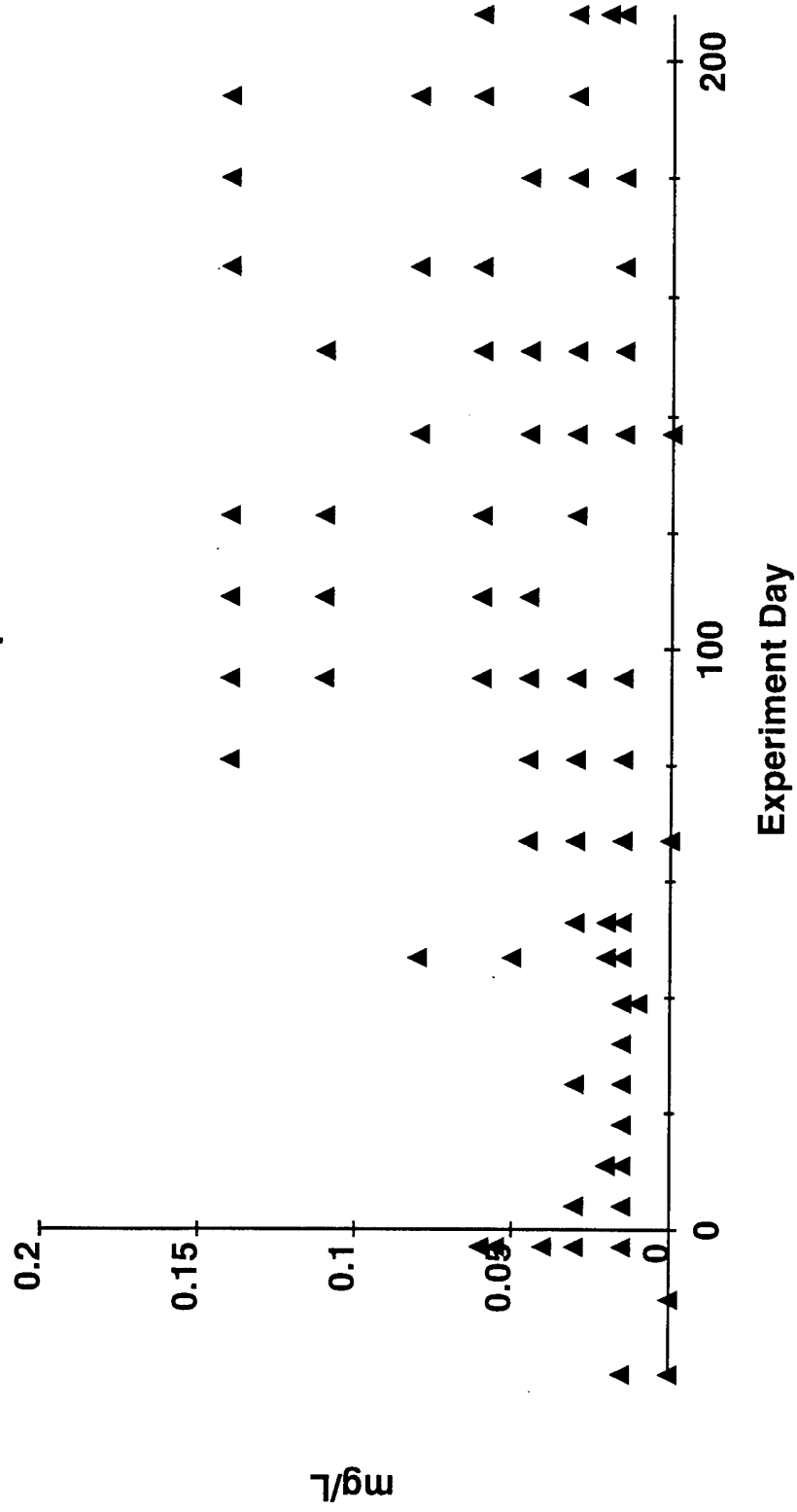
Figure 92d. Water Quality
Soluble Reactive Phosphate - Undosed
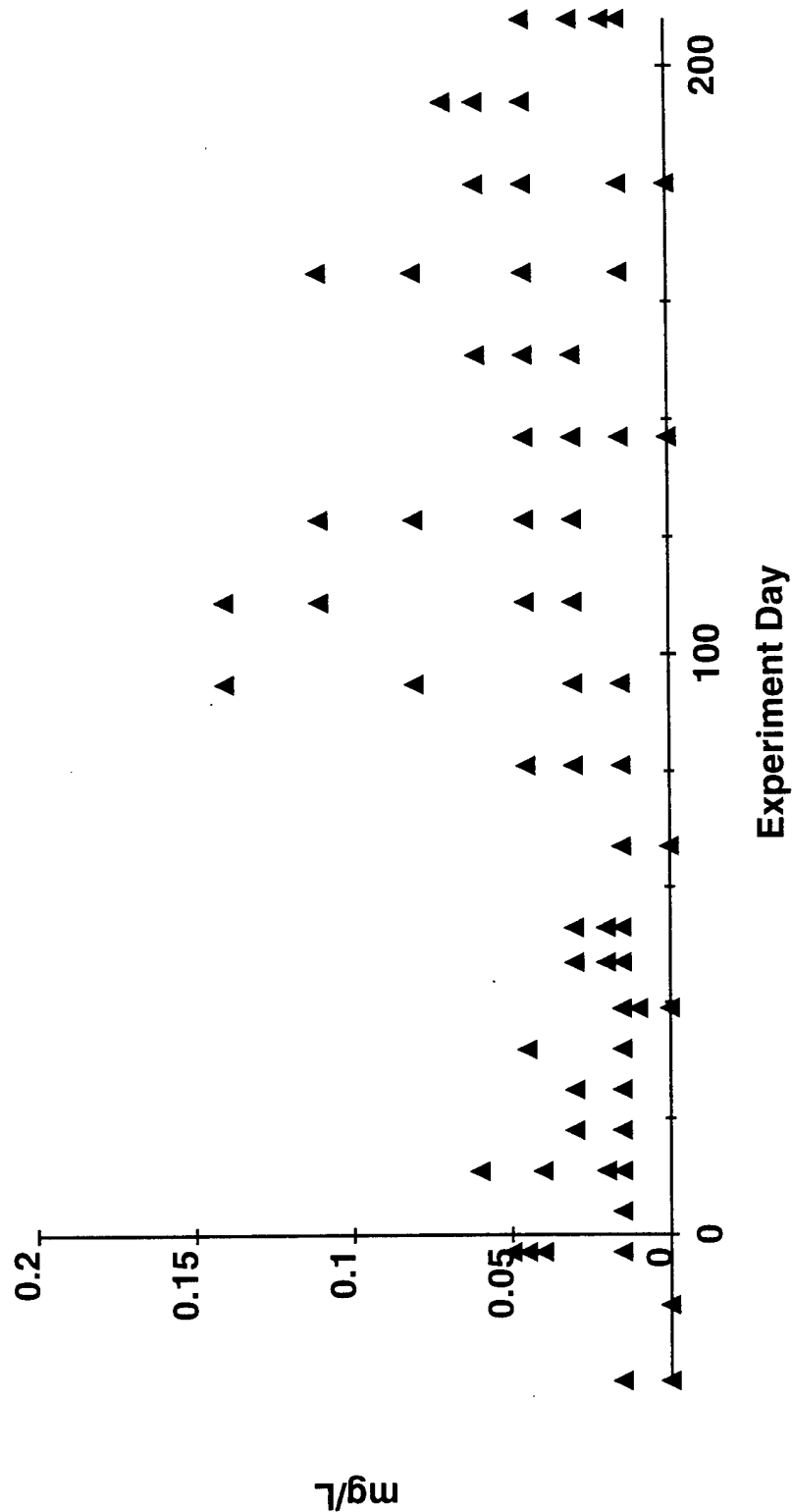
Figure 93a. Water Quality
Photosynthesis (Derived from DO) - High Dose

mg/L
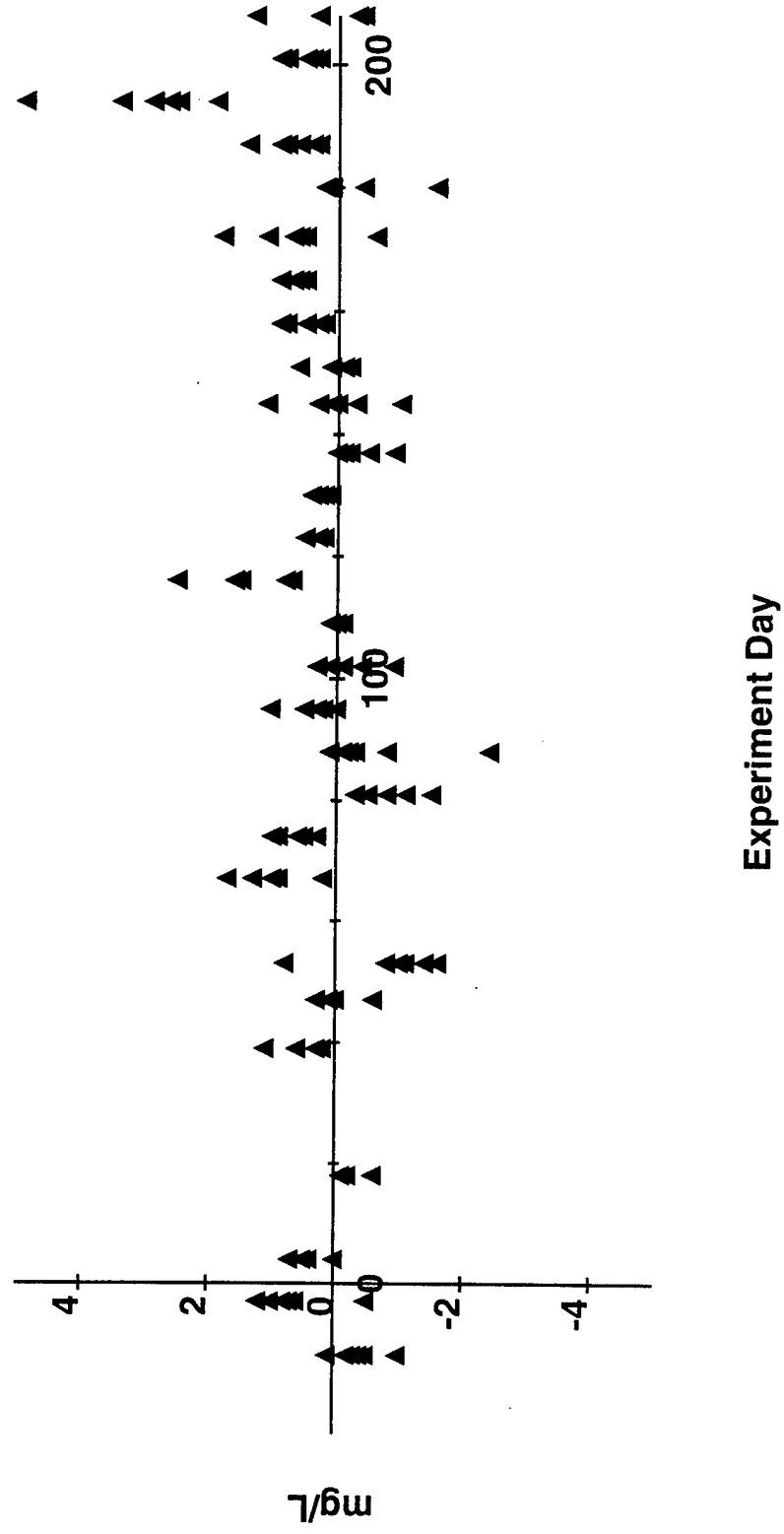
Experiment Day

Figure 93b. Water Quality
Photosynthesis (Derived from DO) - Mid-dose

mg/L
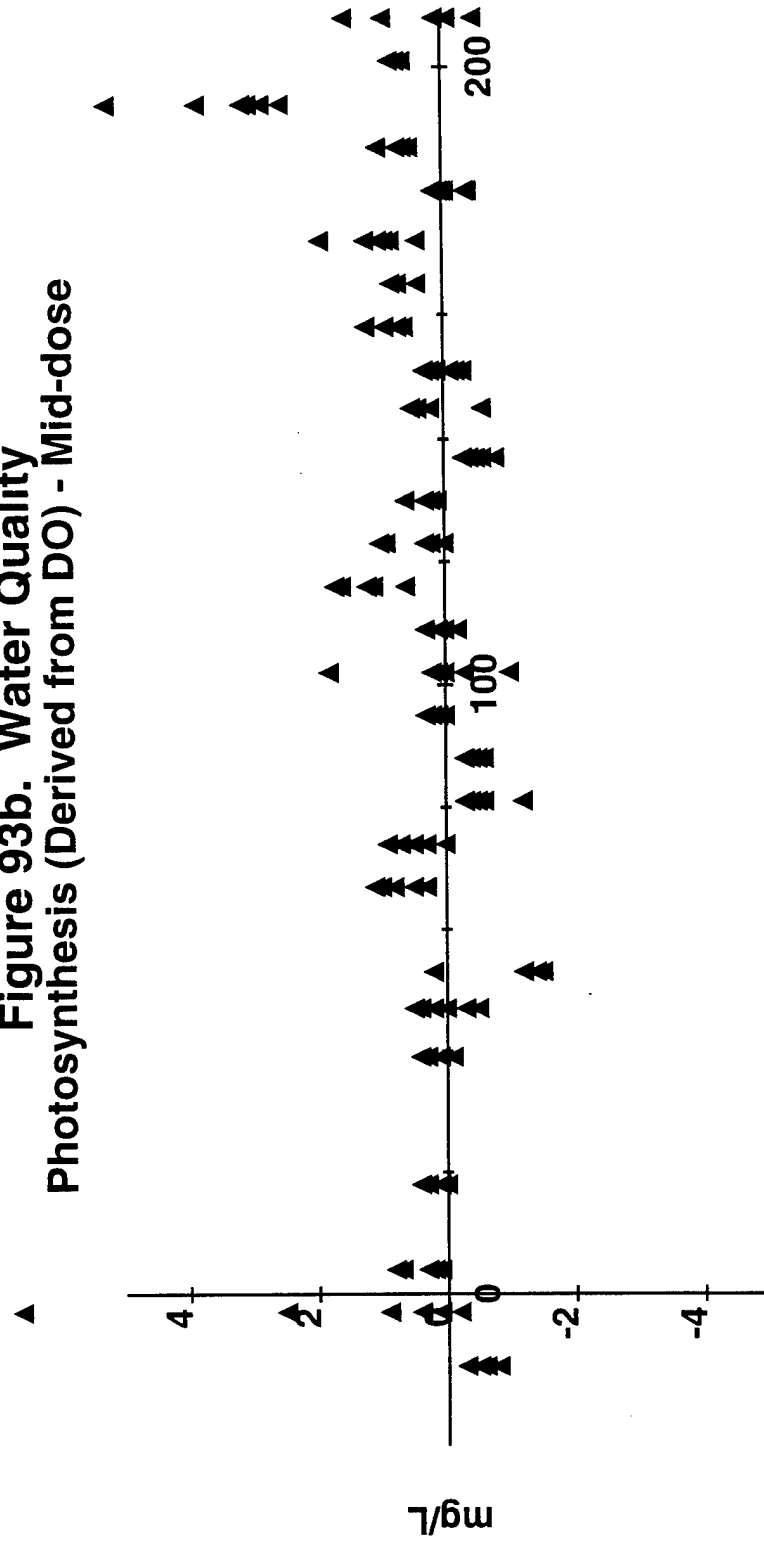
Experiment Day

Figure 93c. Water Quality
Photosynthesis (Derived from DO) - Low Dose

Experiment Day

mg/L
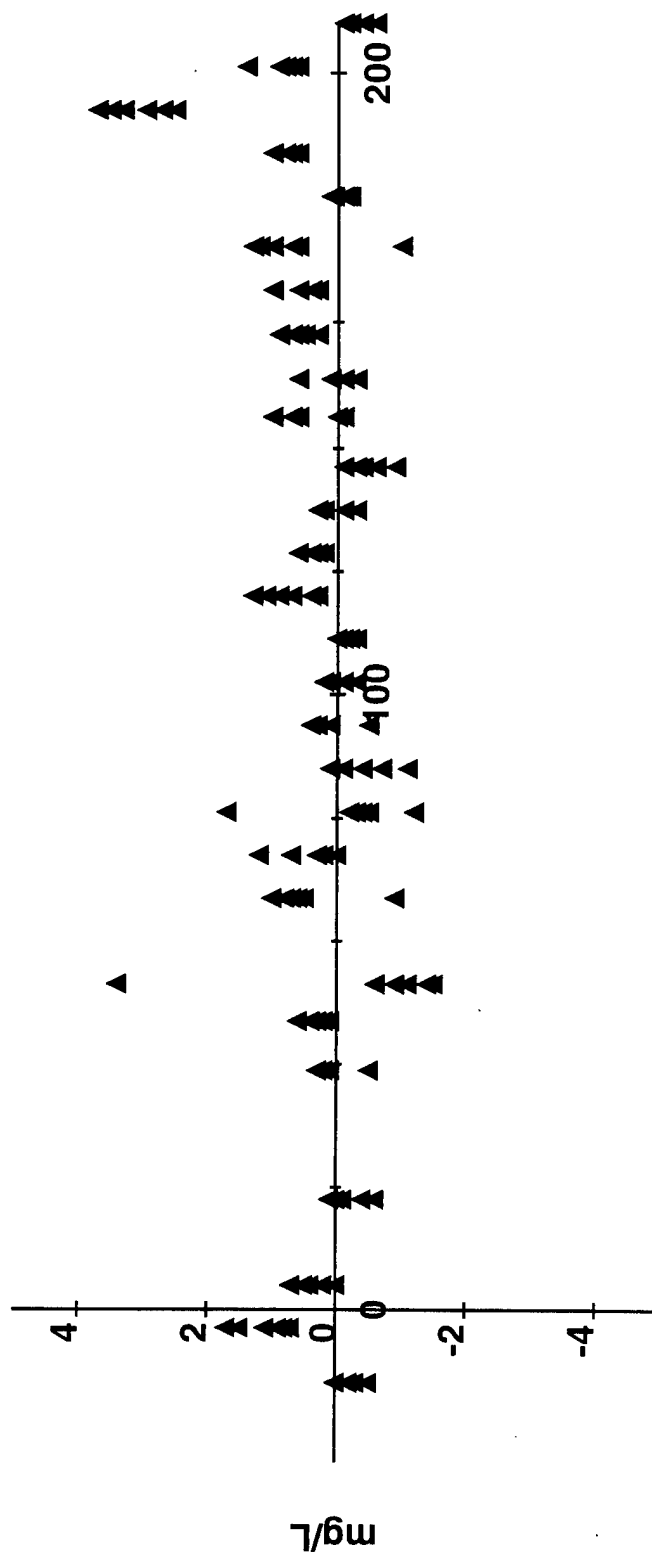
Figure 93d. Water Quality
Photosynthesis (Derived from DO) - Undosed

Experiment Day

mg/L
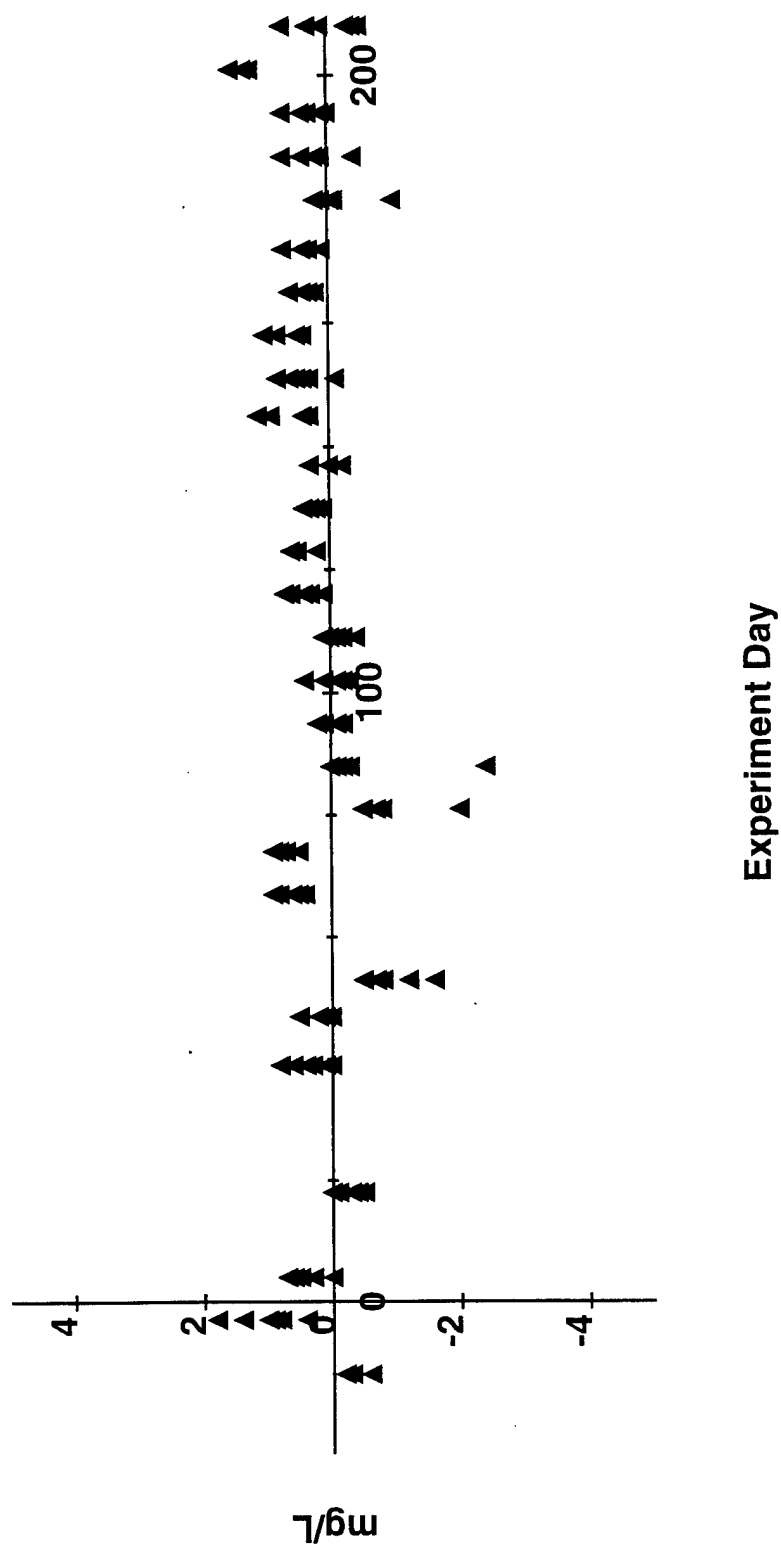
Figure 94a. Water Quality
Respiration (Derived from DO) - High Dose

mg/L
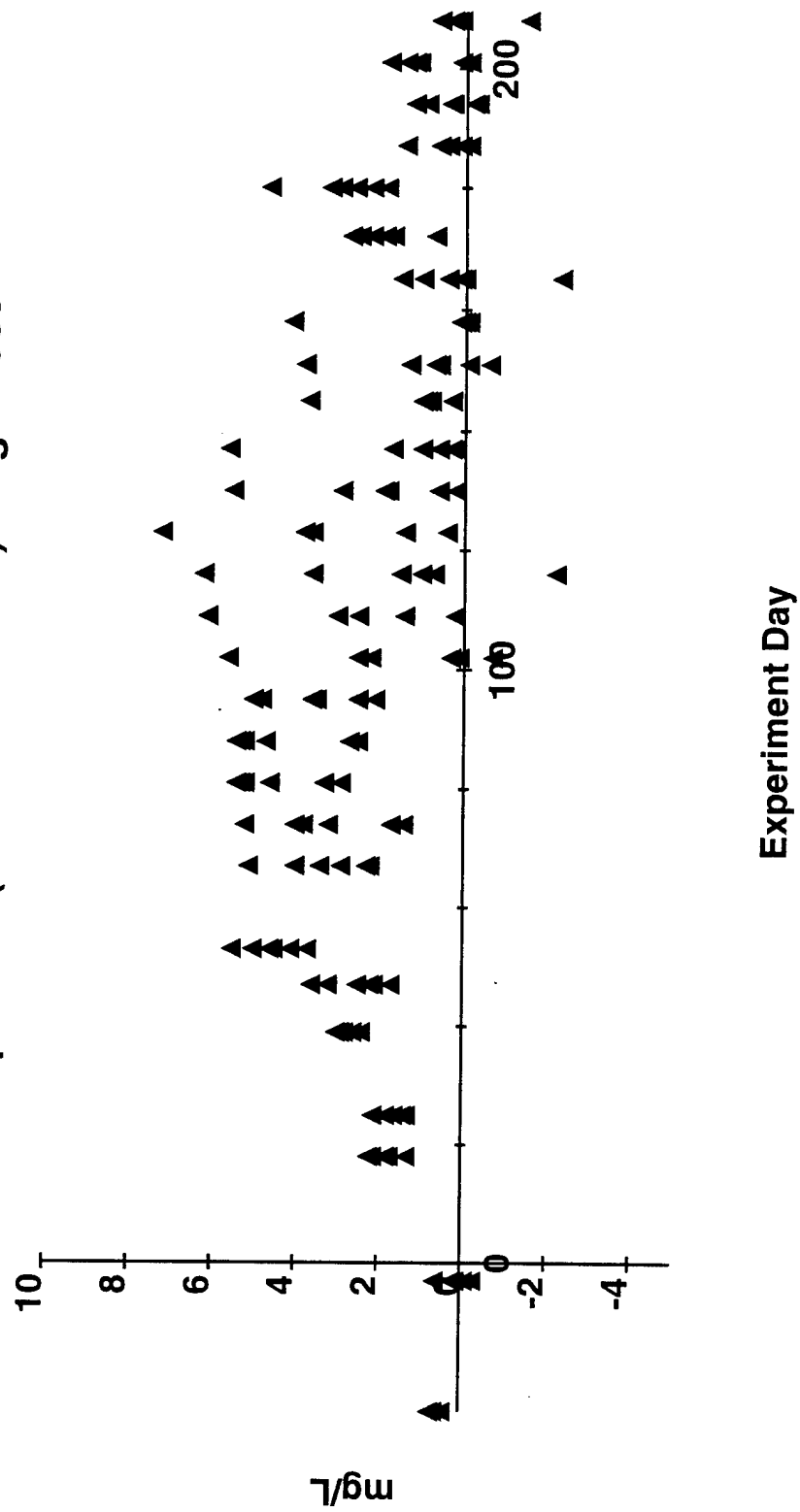
Experiment Day

Figure 94b. Water Quality
Respiration (Derived from DO) - Mid-dose
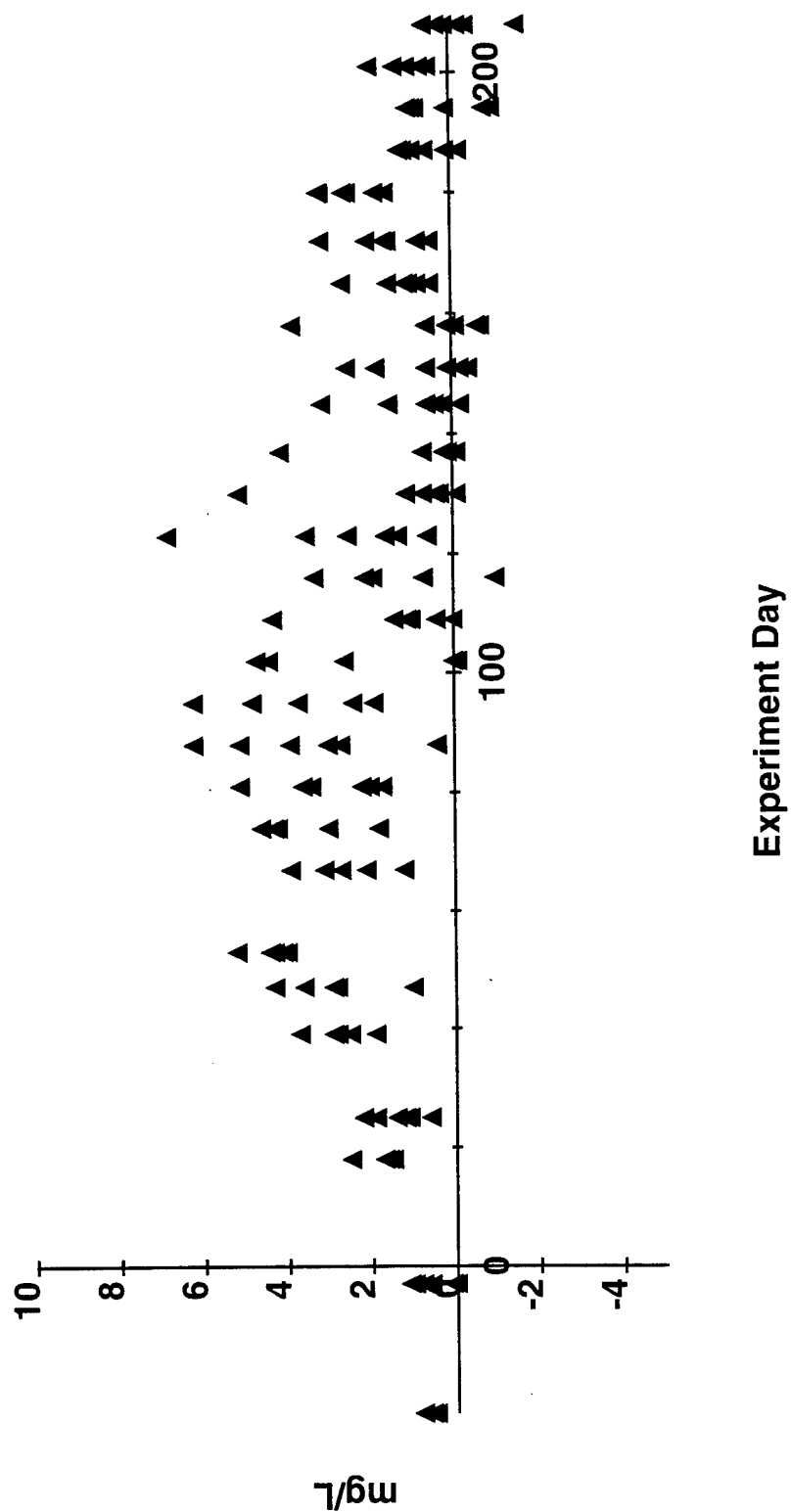
Figure 94c. Water Quality
Respiration (Derived from DO) - Low Dose

mg/L
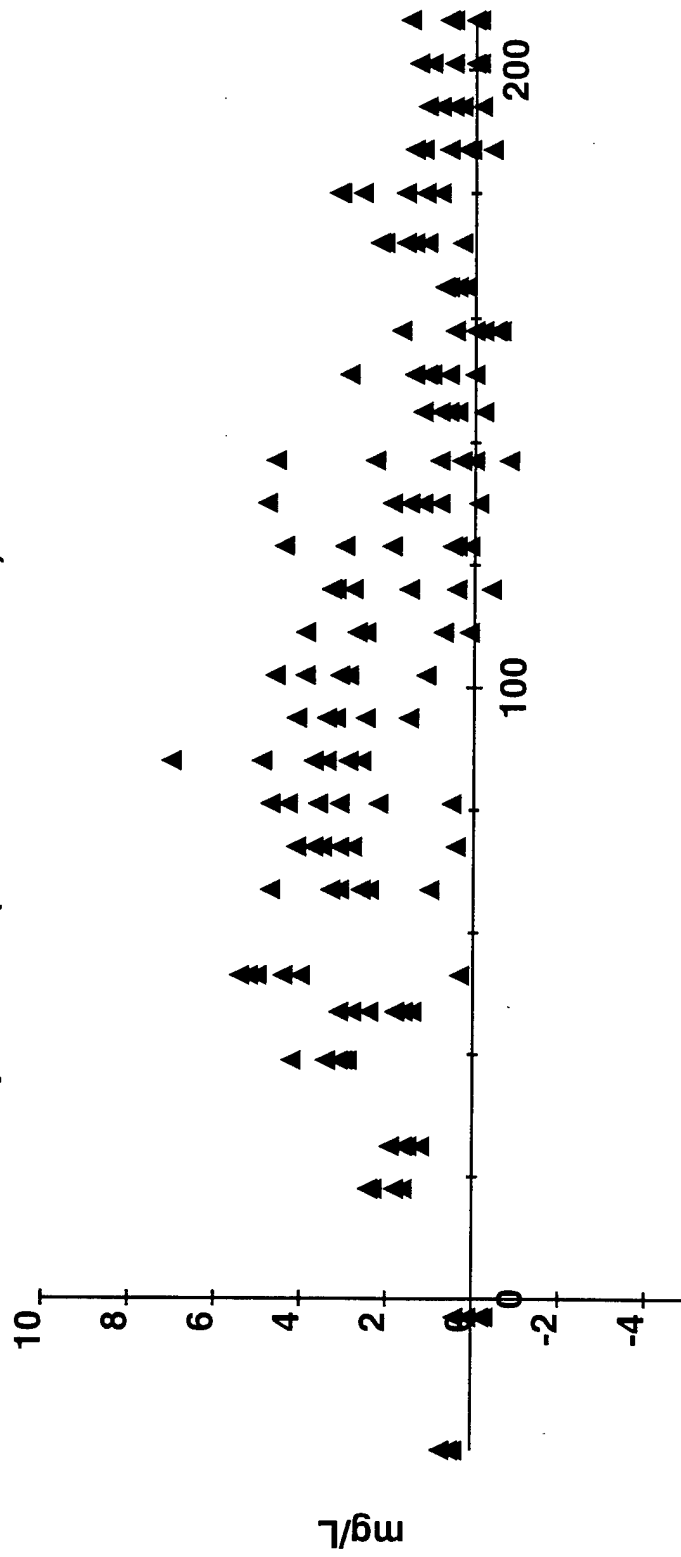
Experiment Day

Figure 94d. Water Quality
Respiration (Derived from DO) - Undosed

Experiment Day
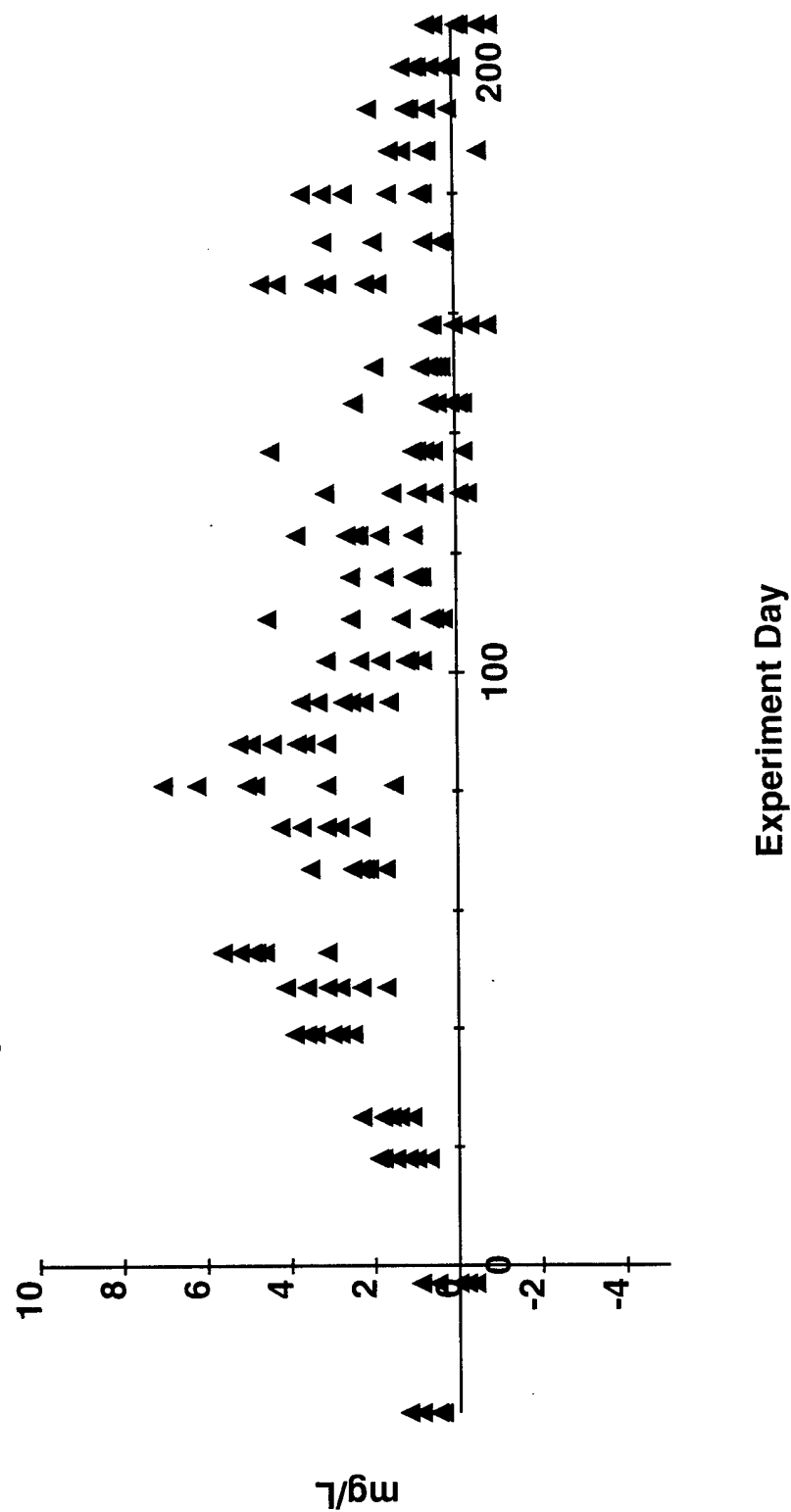
Figure 95a. Water Quality
Turbidity - High Dose

NTU

0

10

20

0

100

200

Experiment Day
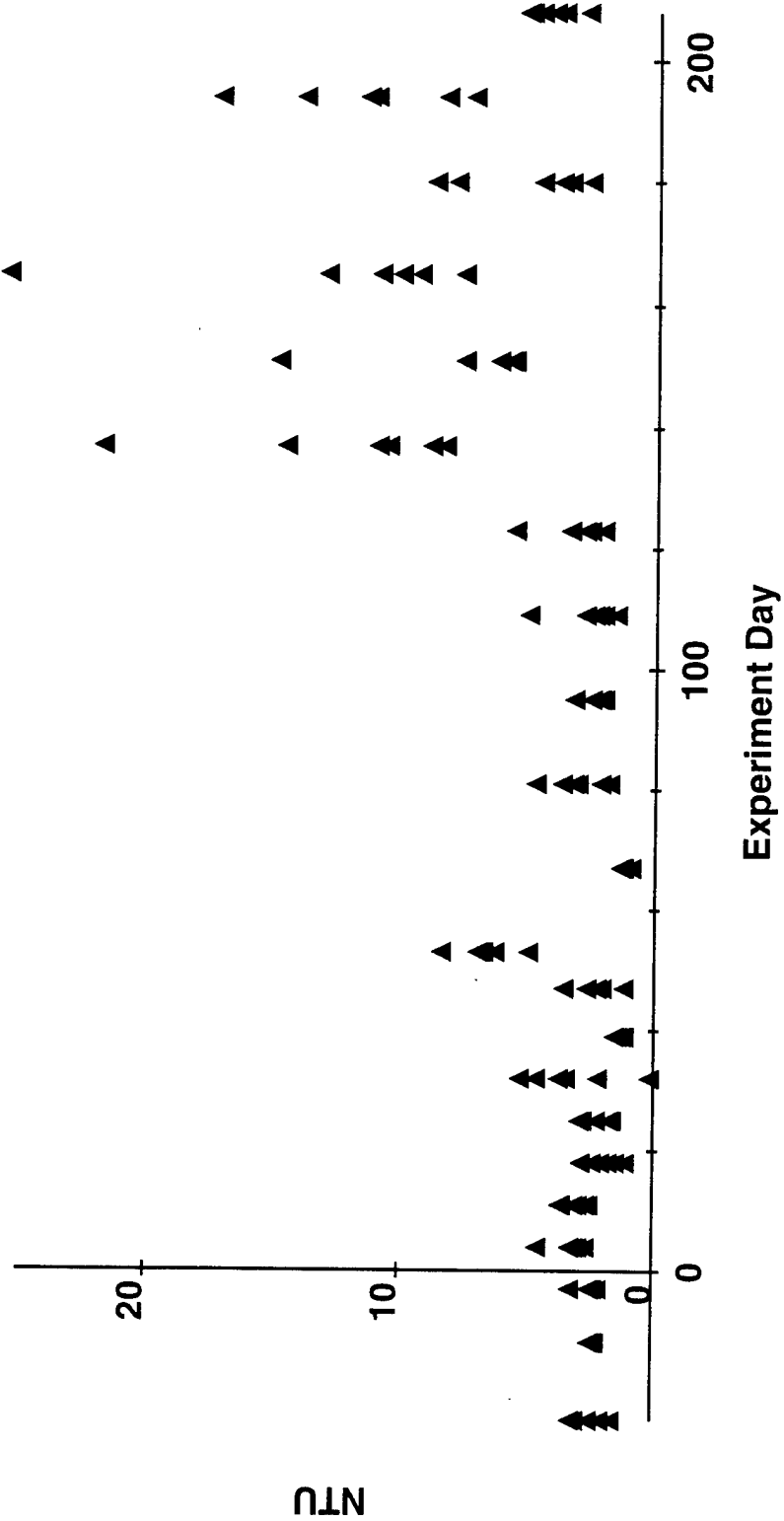
Figure 95b. Water Quality
Turbidity - Mid-dose
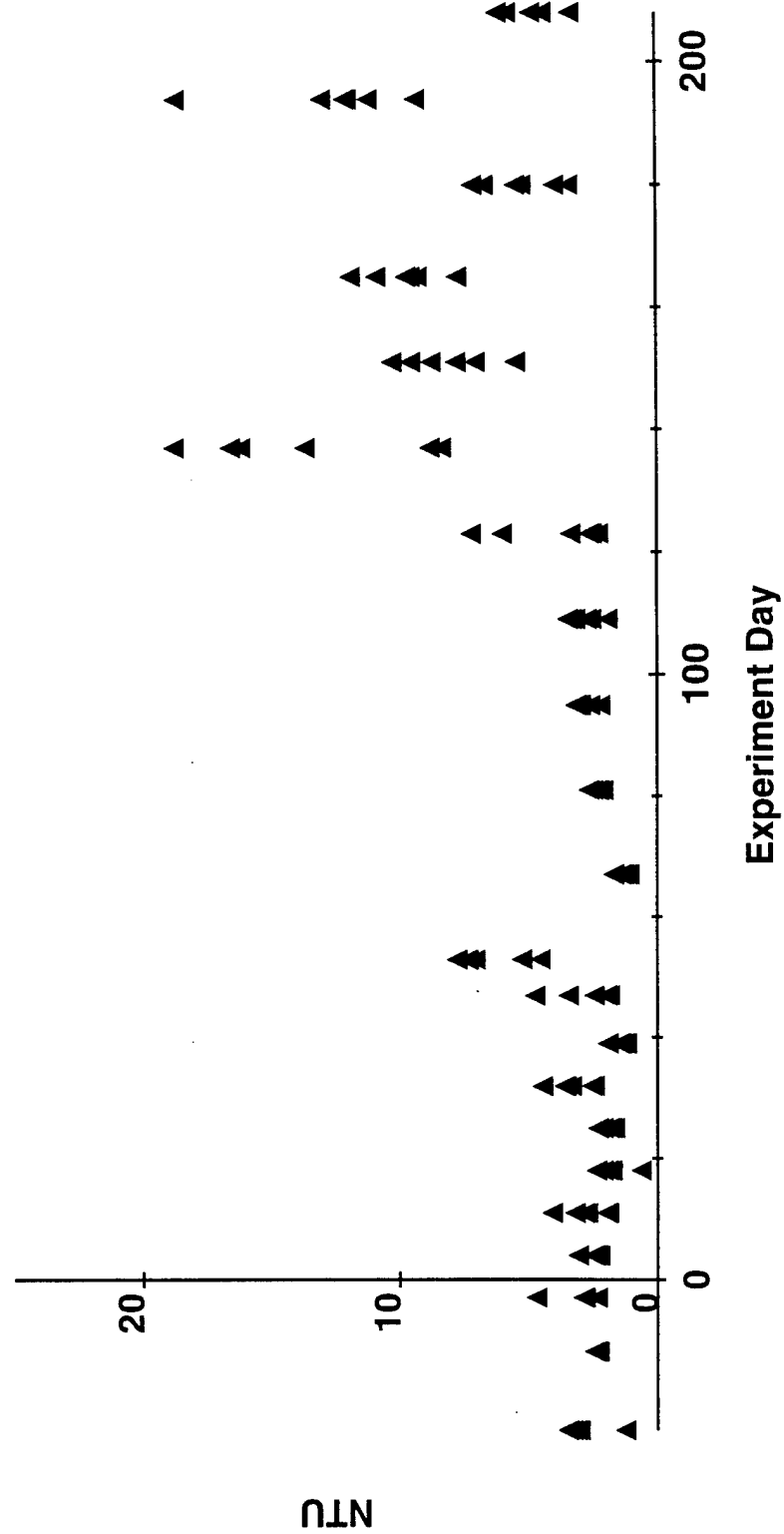
Figure 95c. Water Quality
Turbidity - Low Dose
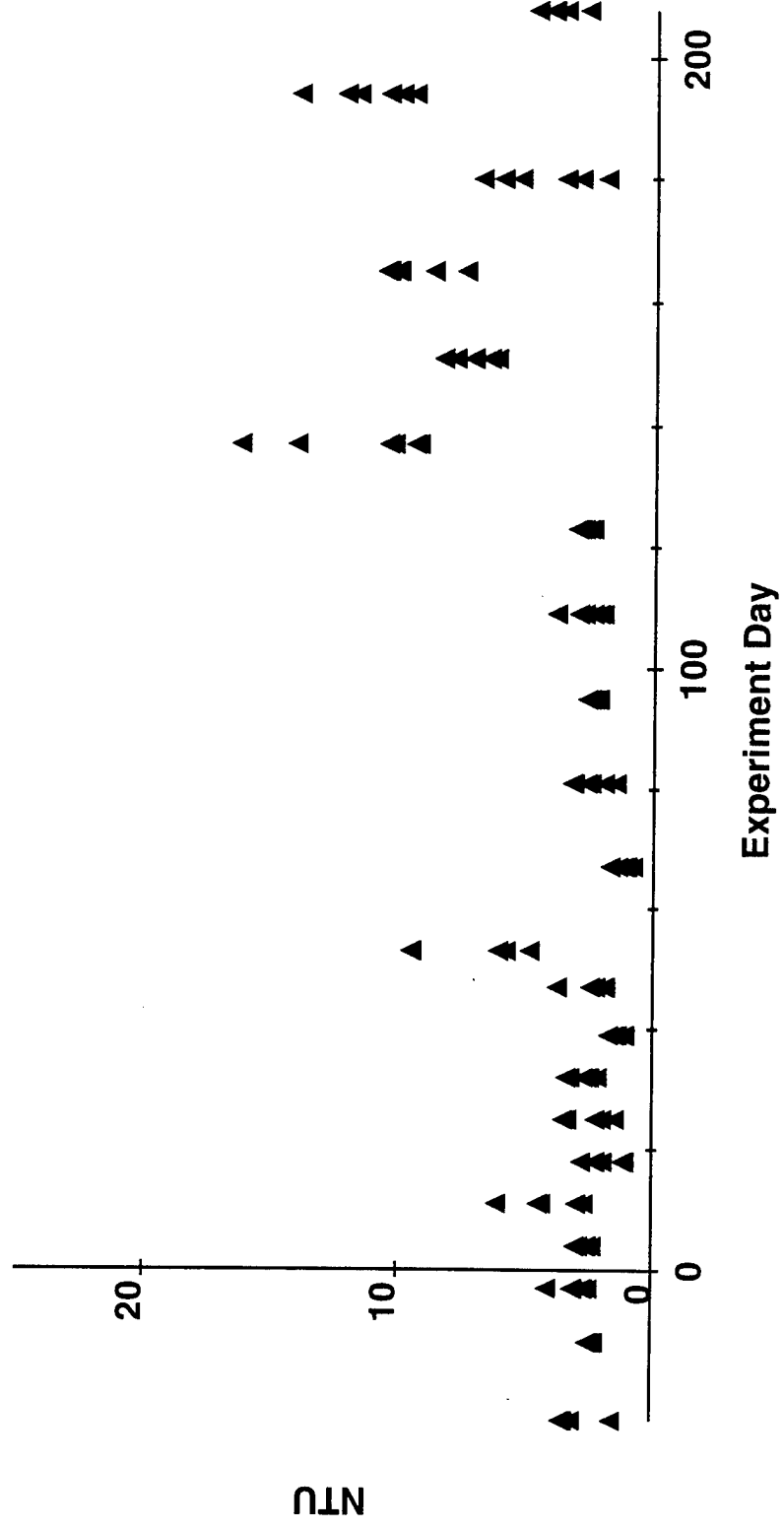
Figure 95d. Water Quality
Turbidity - Undosed
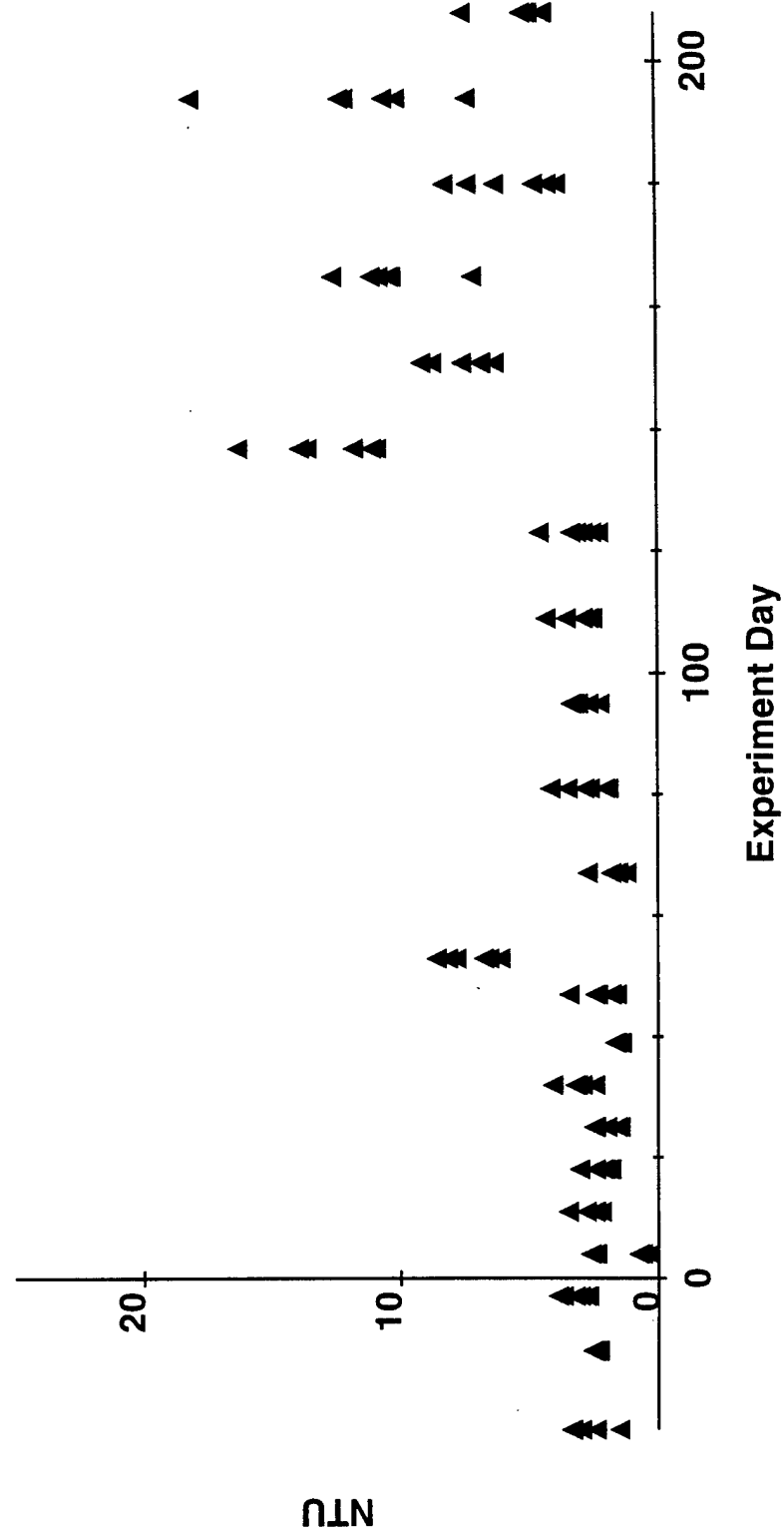
Figure 96a. Water Quality
Water Temperature - High Dose
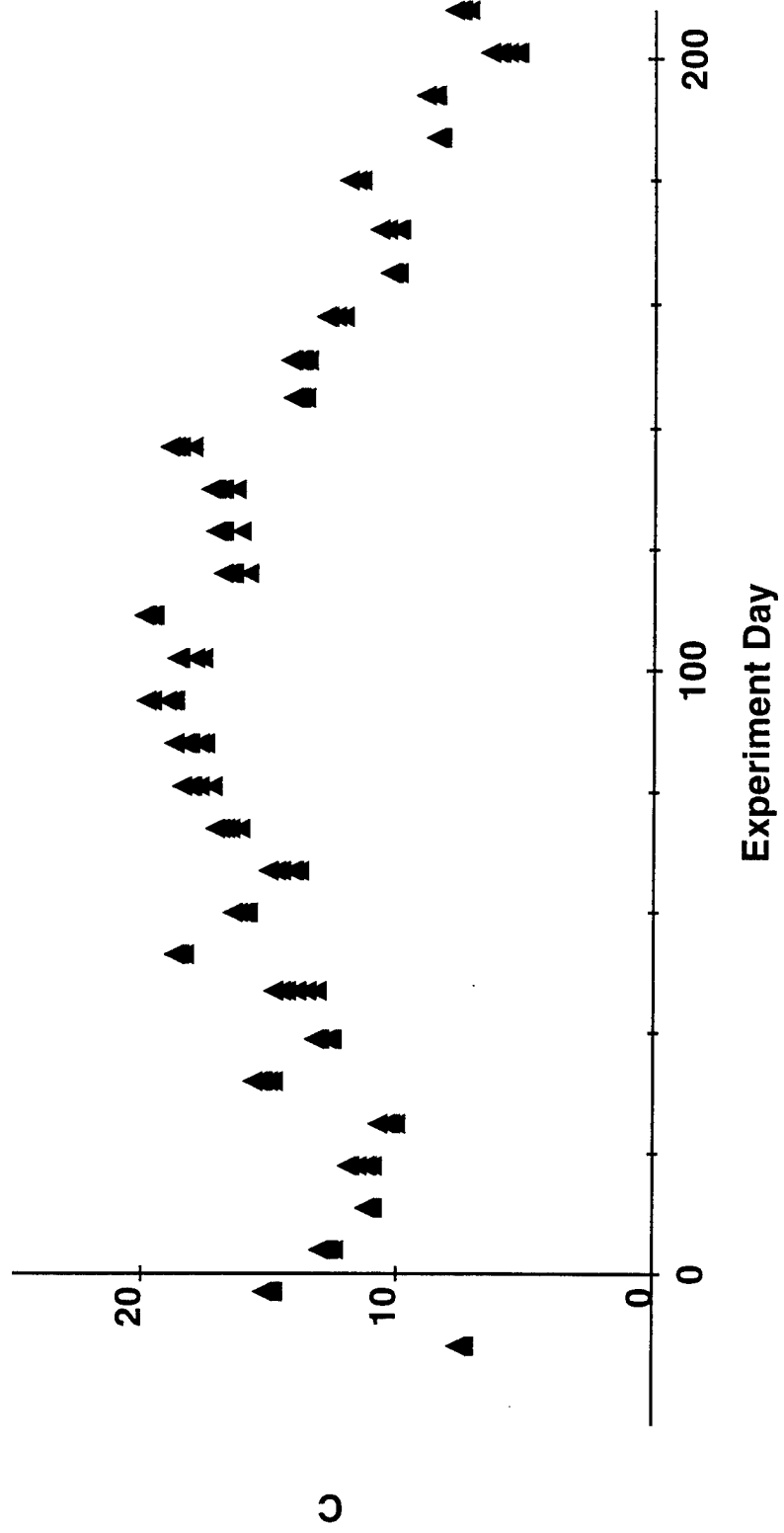
Figure 96b. Water Quality
Water Temperature - Mid-dose
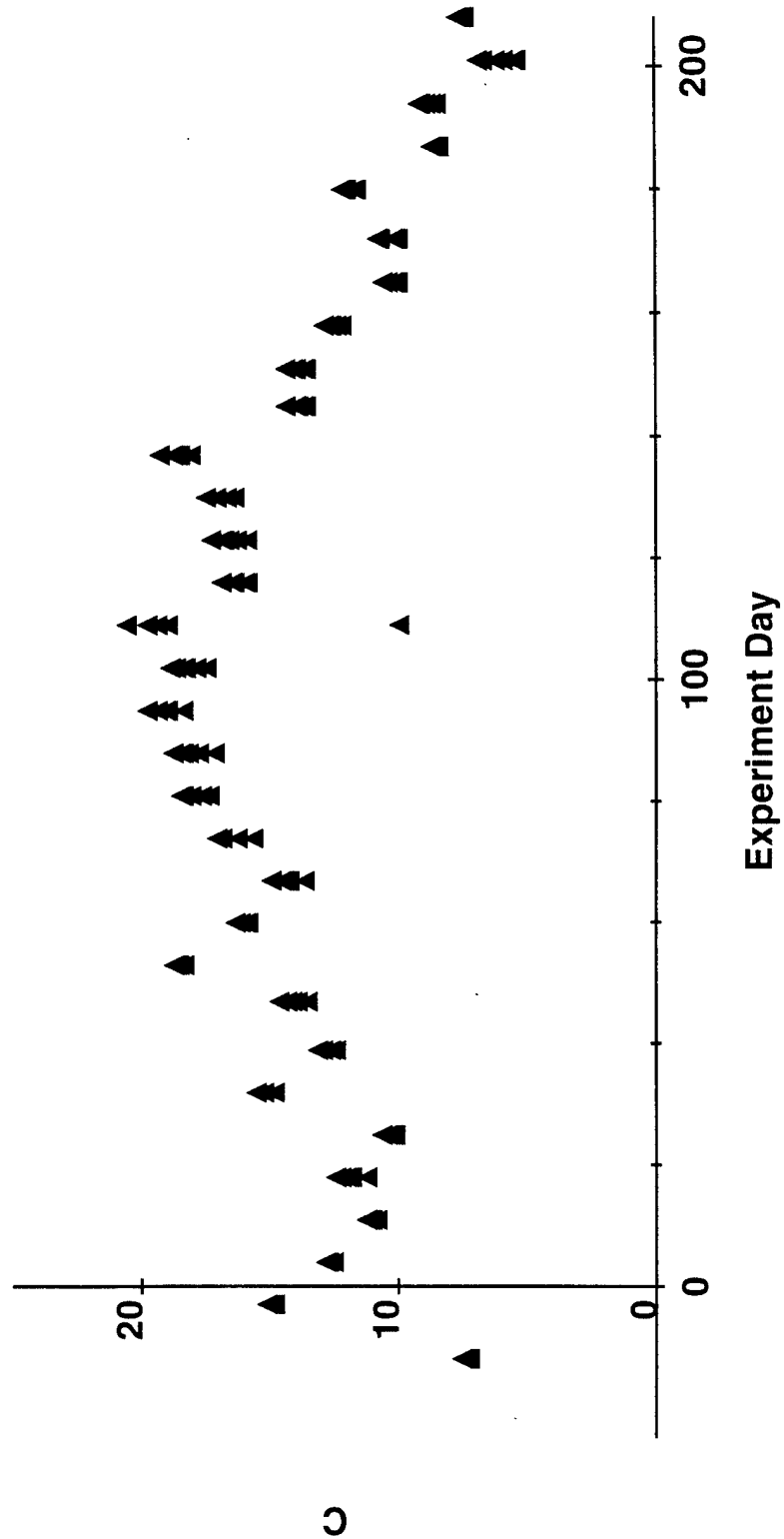
Figure 96c. Water Quality
Water Temperature - Low Dose
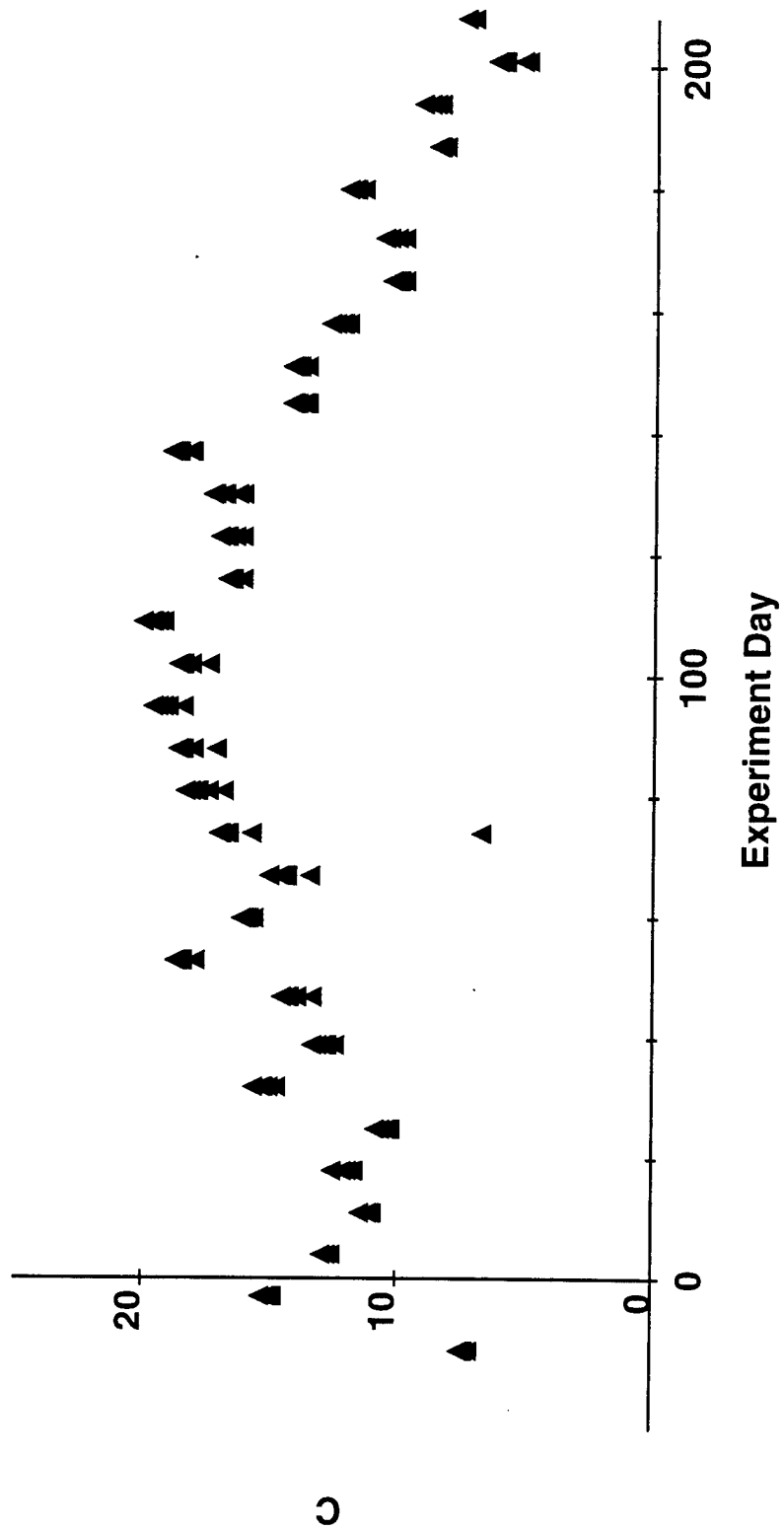
Figure 96d. Water Quality
Water Temperature - Undosed
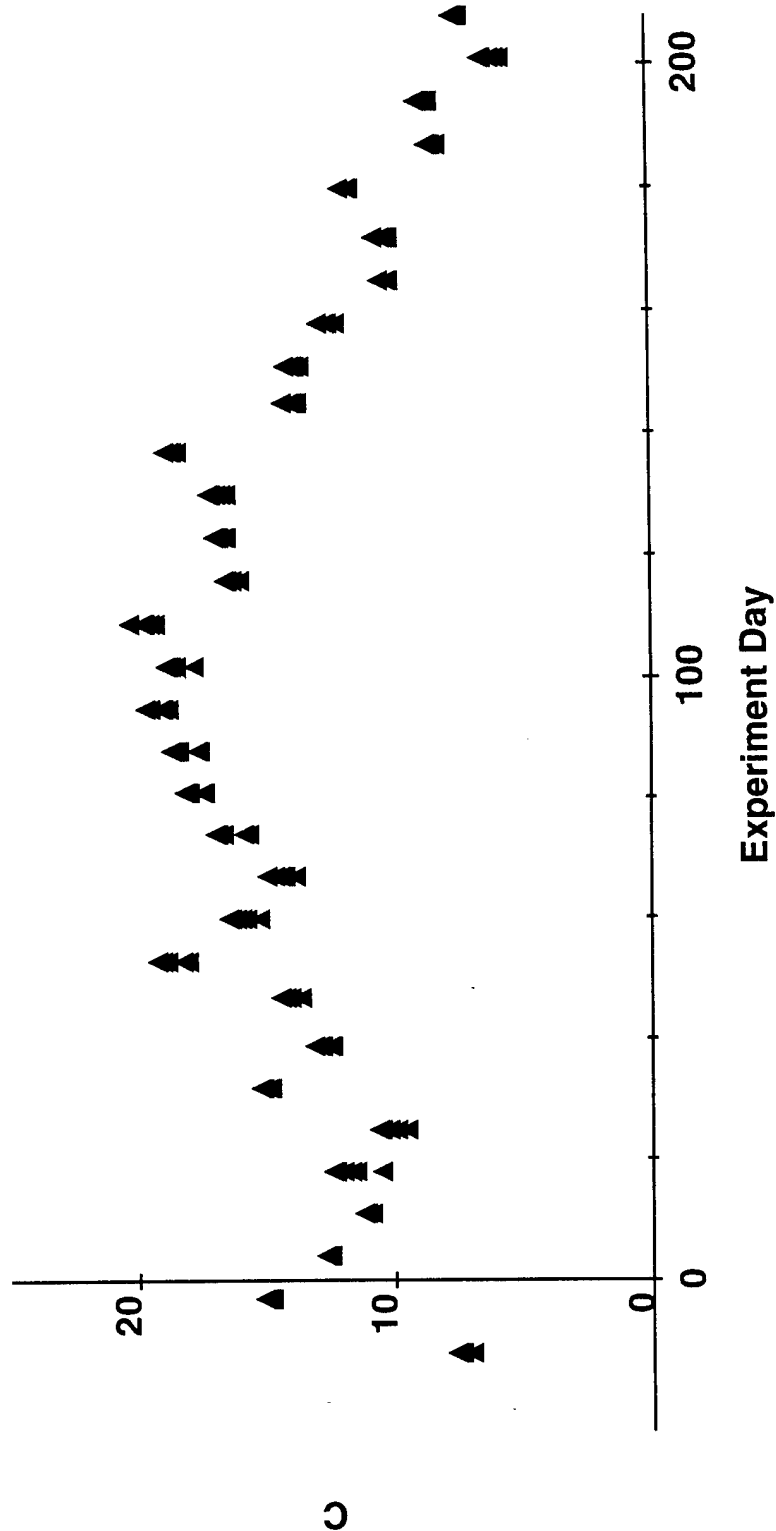
Figure 97. Phytoplankton and Water Quality Correlations
Ankistrodesmus vs. Schroederia
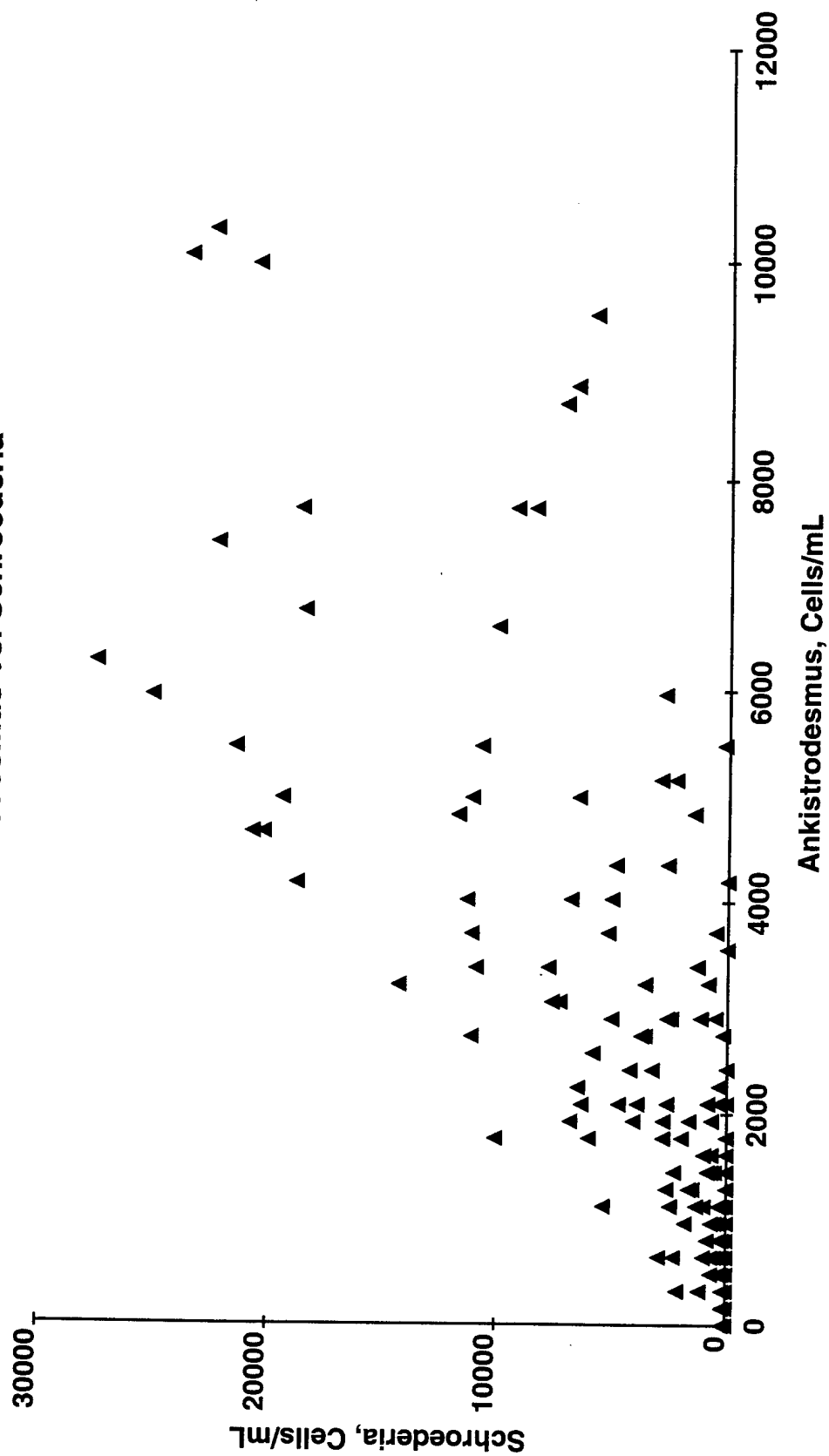
Figure 98. Phytoplankton and Water Quality Correlations
Ankistrodesmus vs. Pennate Diatoms
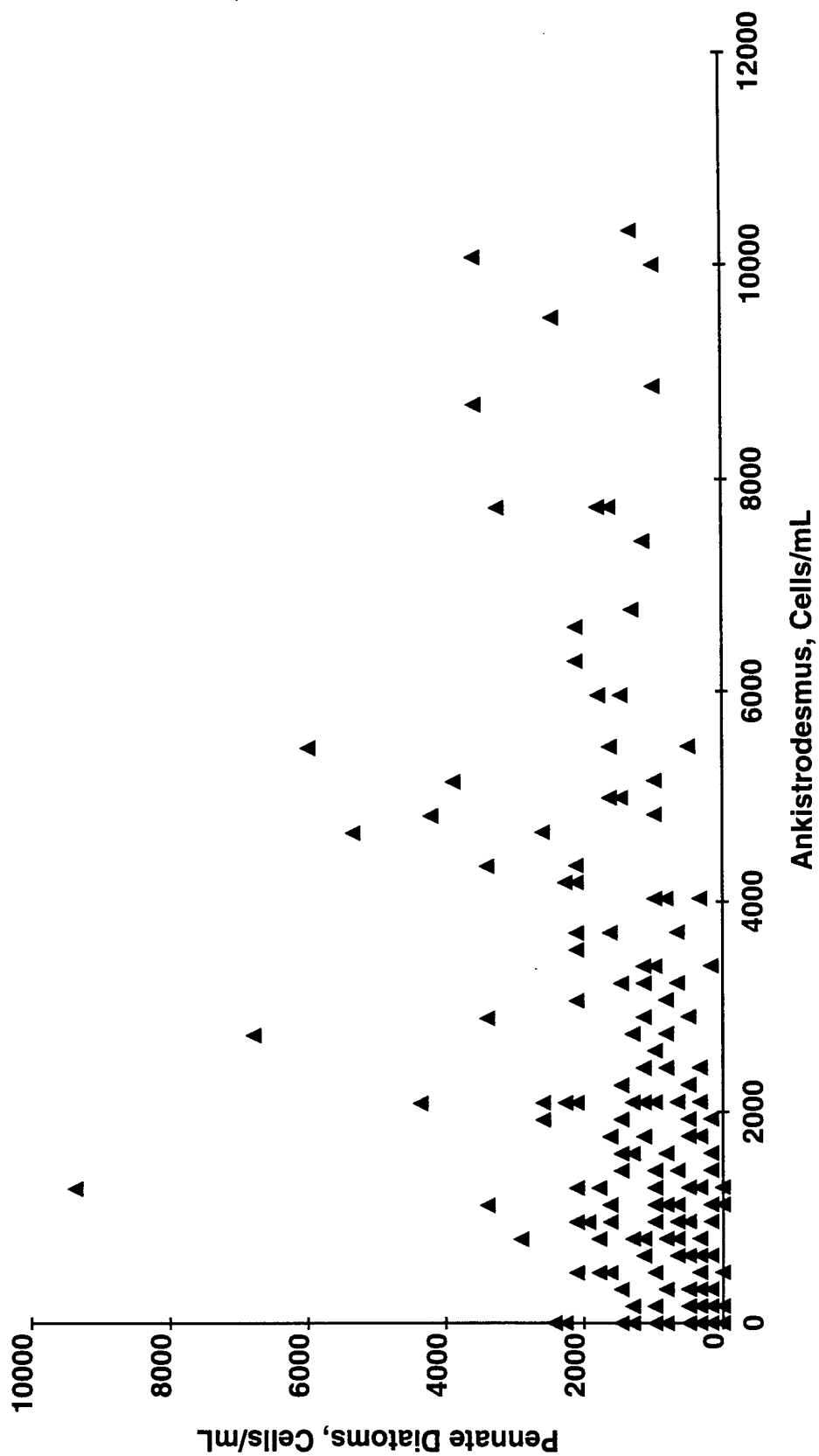
Figure 99. Phytoplankton and Water Quality Correlations
Ankistrodesmus vs. Heterotrophic Flagellates
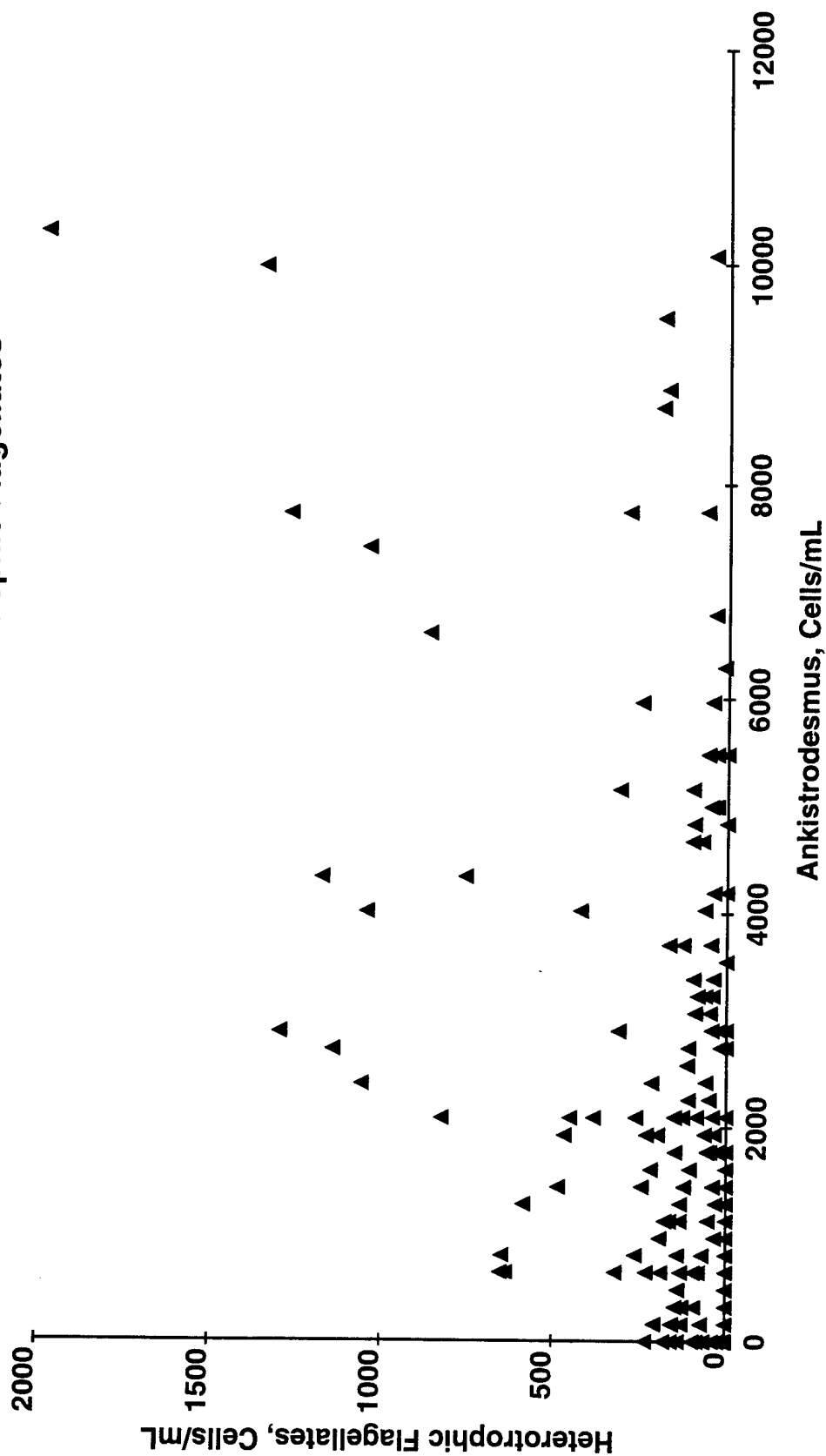
Figure 100. Phytoplankton and Water Quality Correlations
Ankistrodesmus vs. TTC Respiration
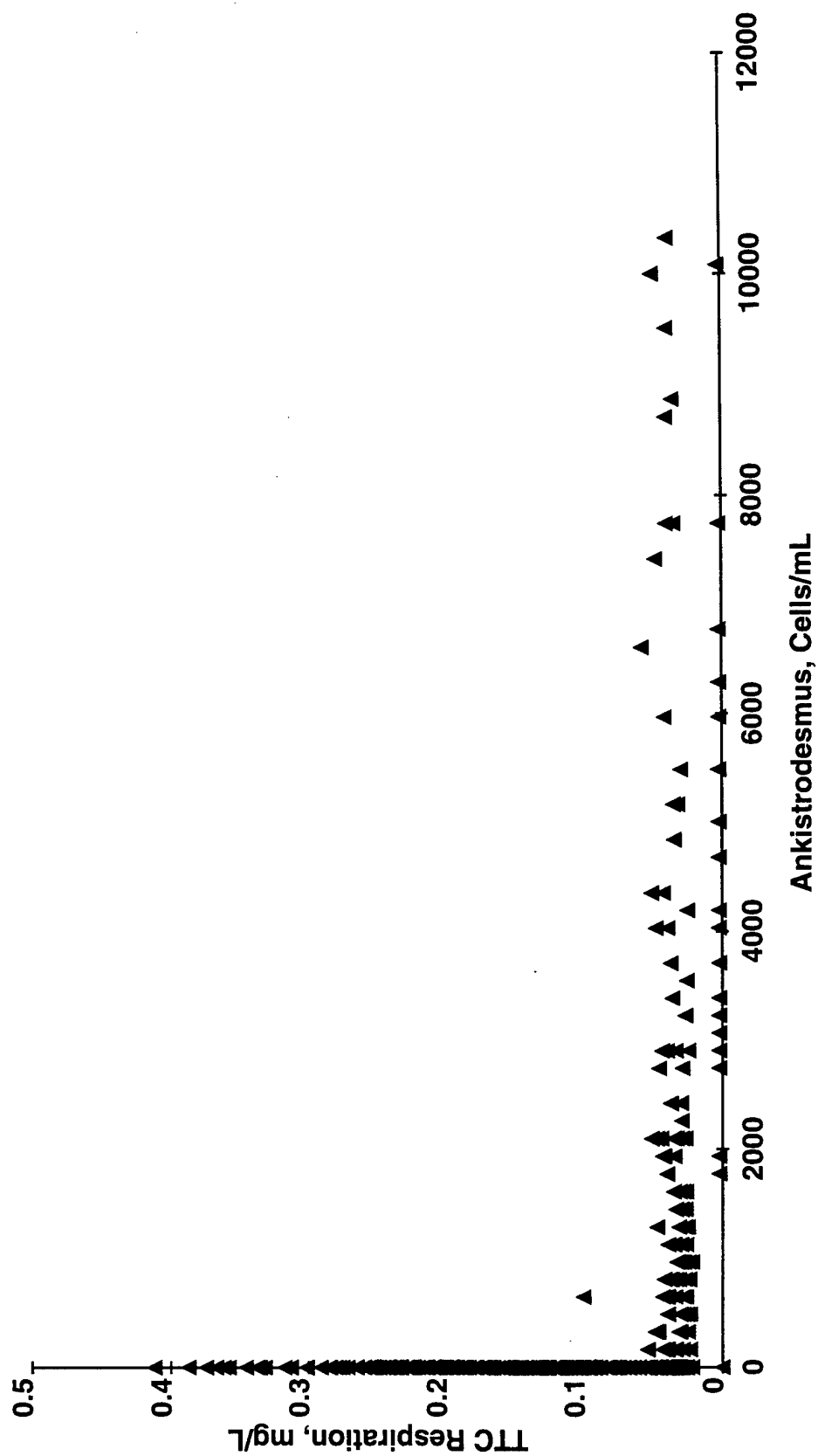
Figure 101. Phytoplankton and Water Quality Correlations
Conductivity vs. Heterotrophic Flagellates
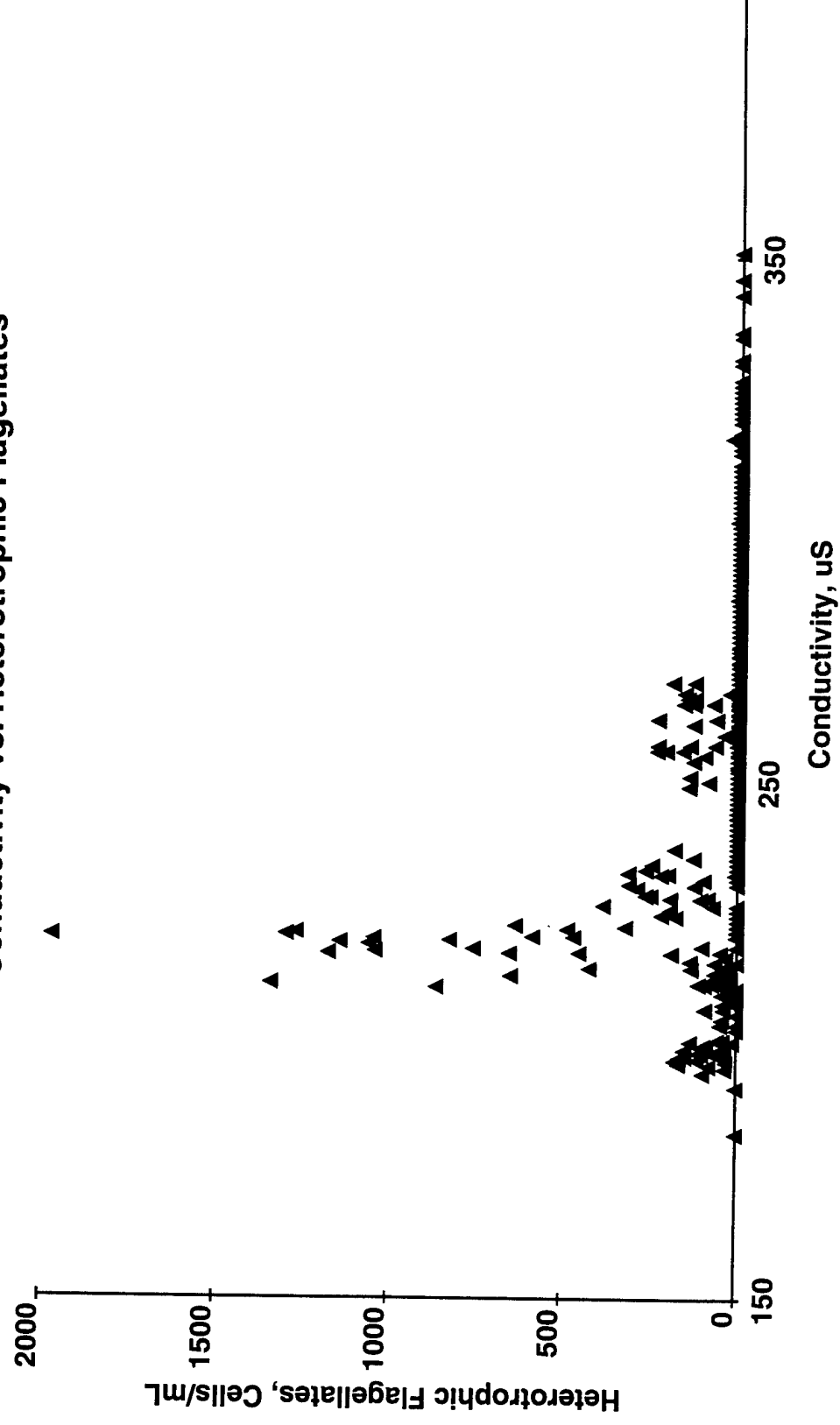
Figure 102. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO1 vs. pmDO2
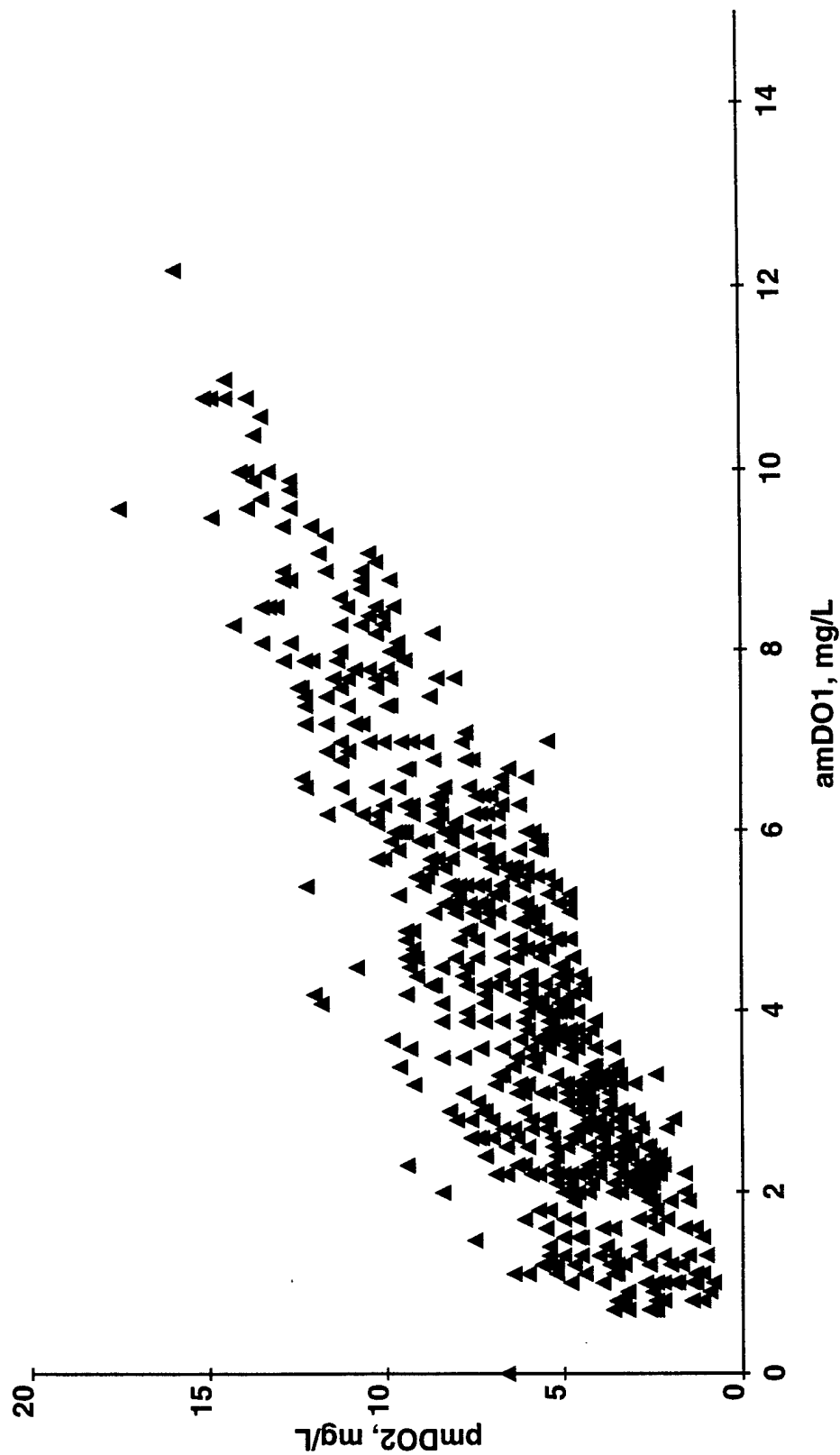
Figure 103. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO1 vs. amDO3
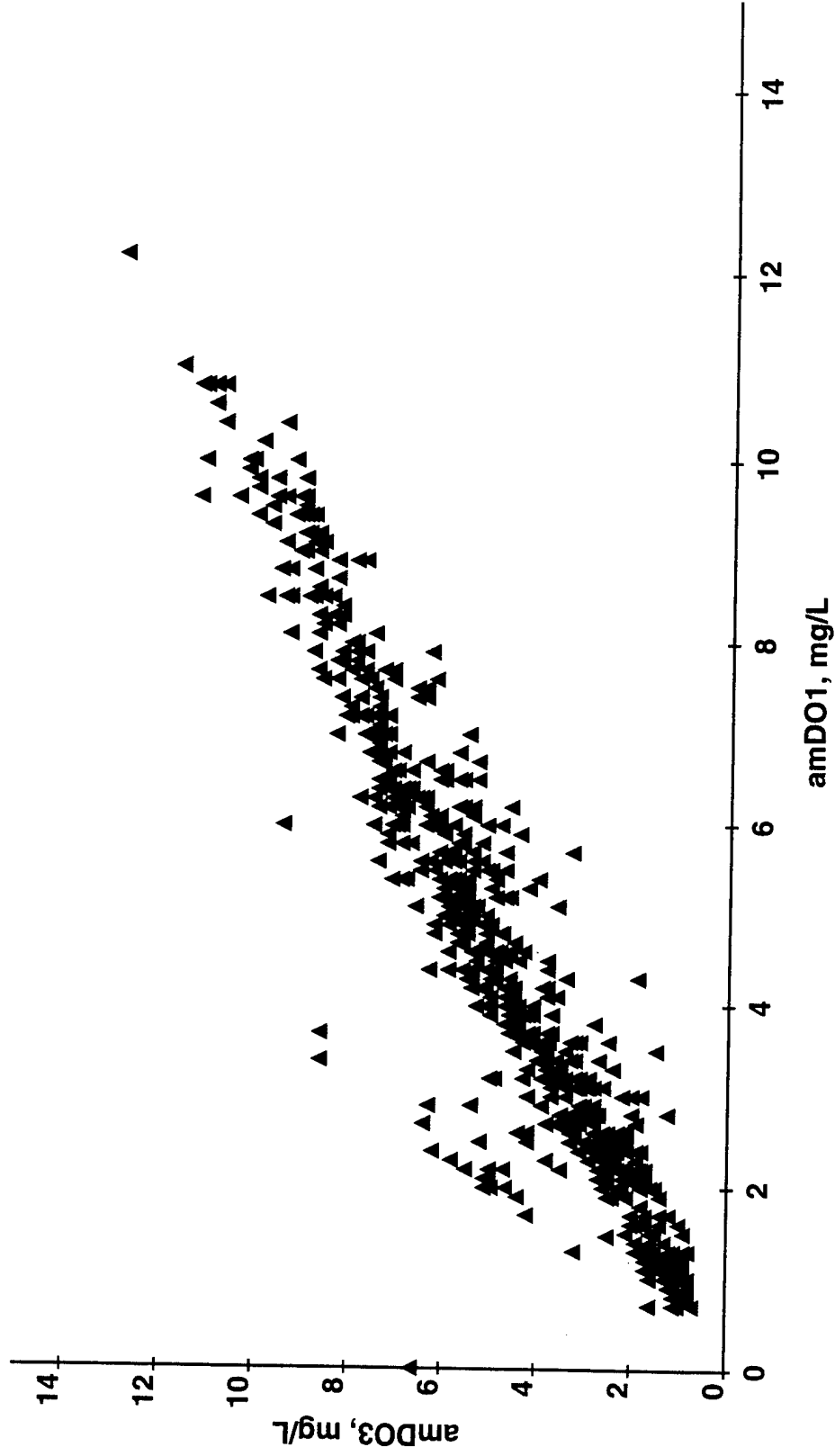
Figure 104. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO1 vs. Soluble Reactive Phosphate
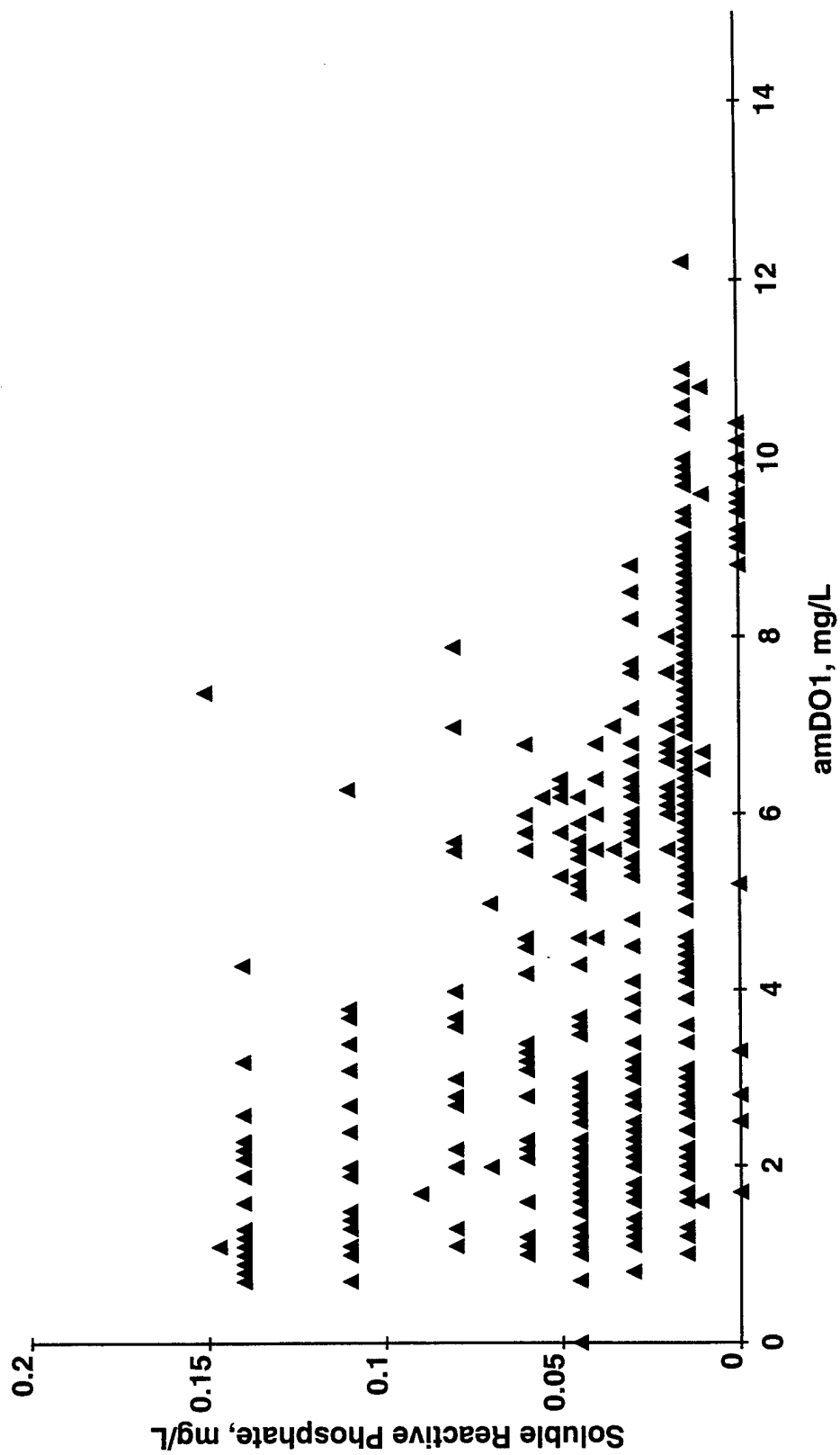
Figure 105. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO1 vs. TTC Respiration
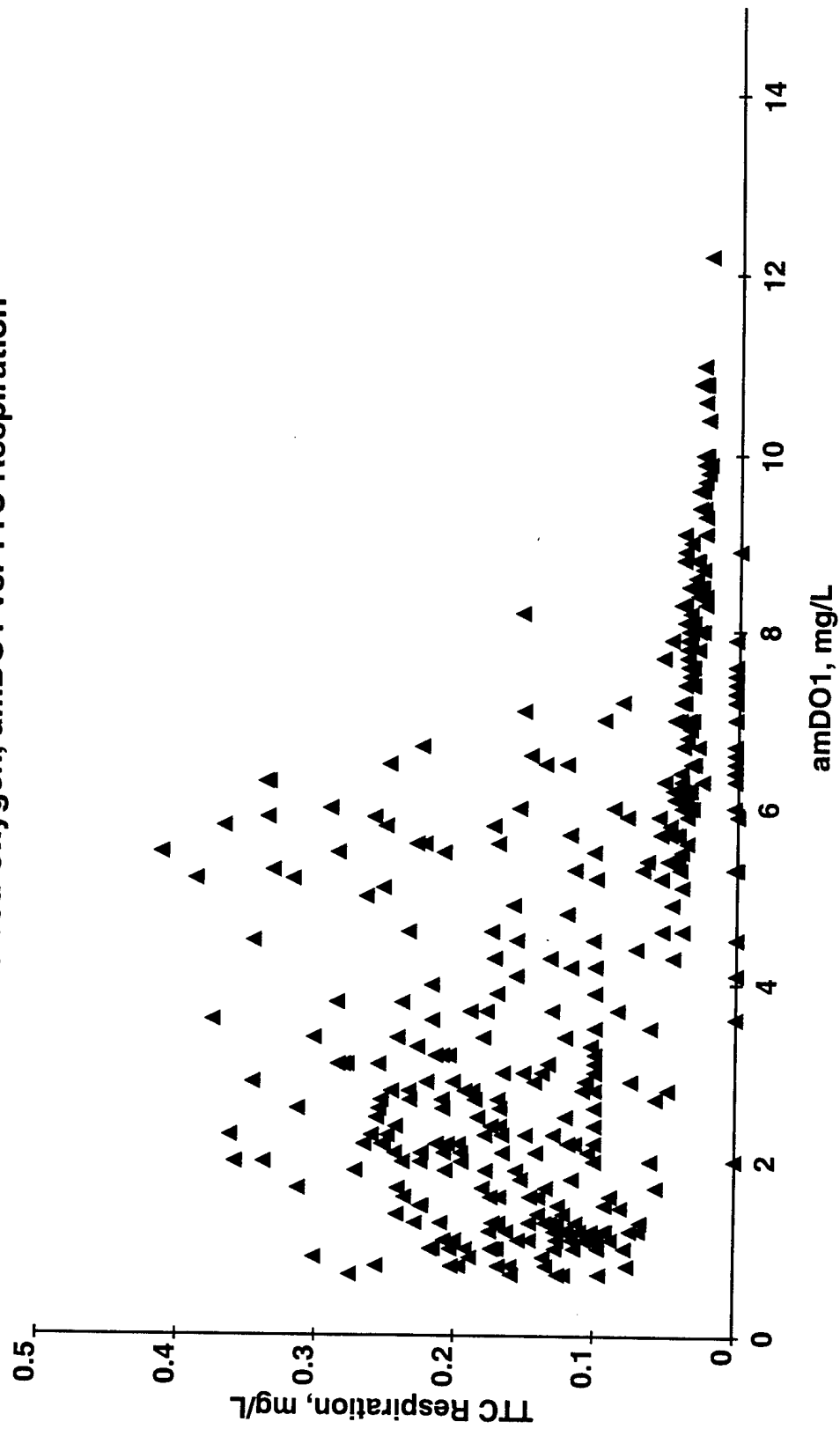
Figure 106. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, pmDO2 vs. amDO3
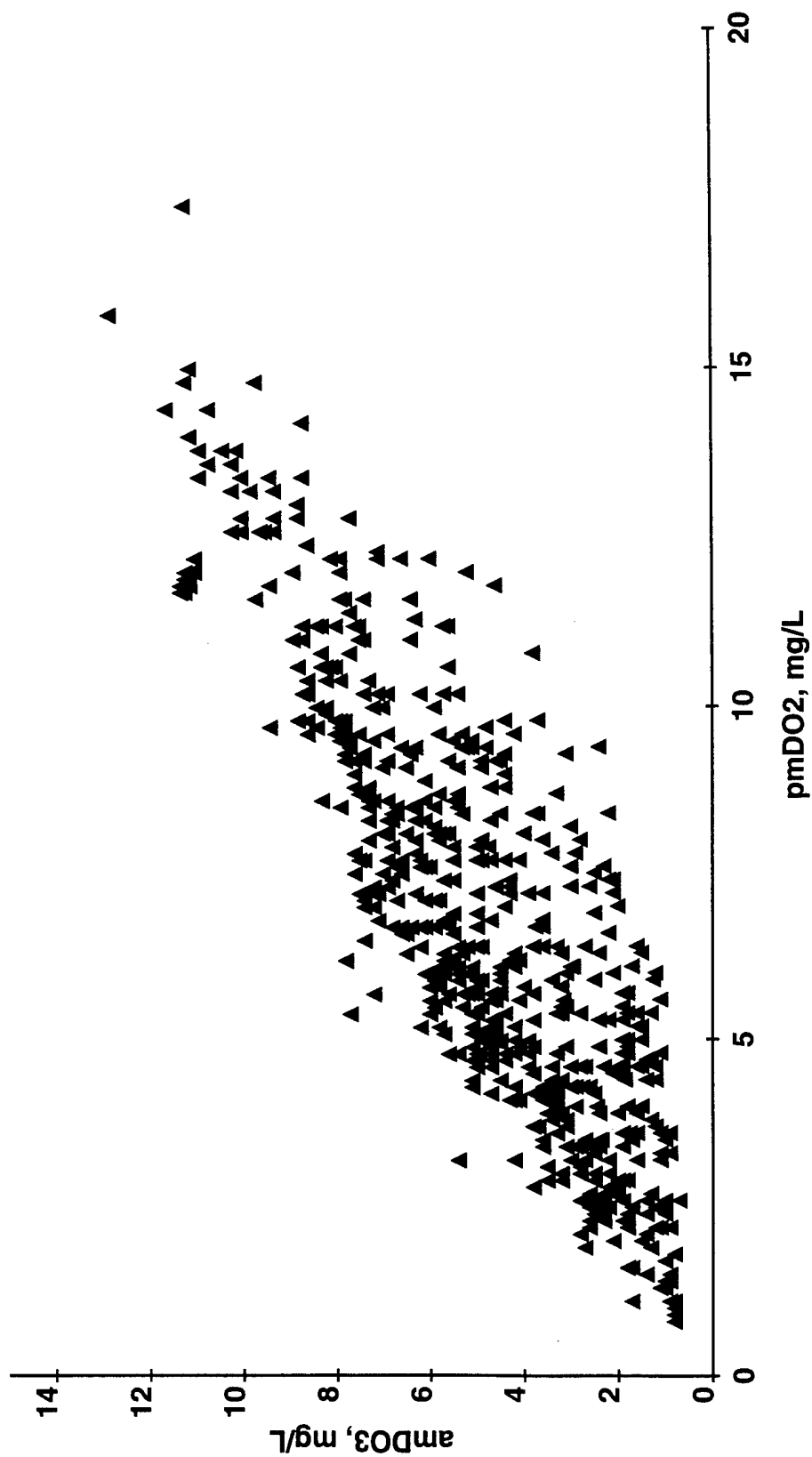
Figure 107. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, pmDO2 vs. Soluble Reactive Phosphate
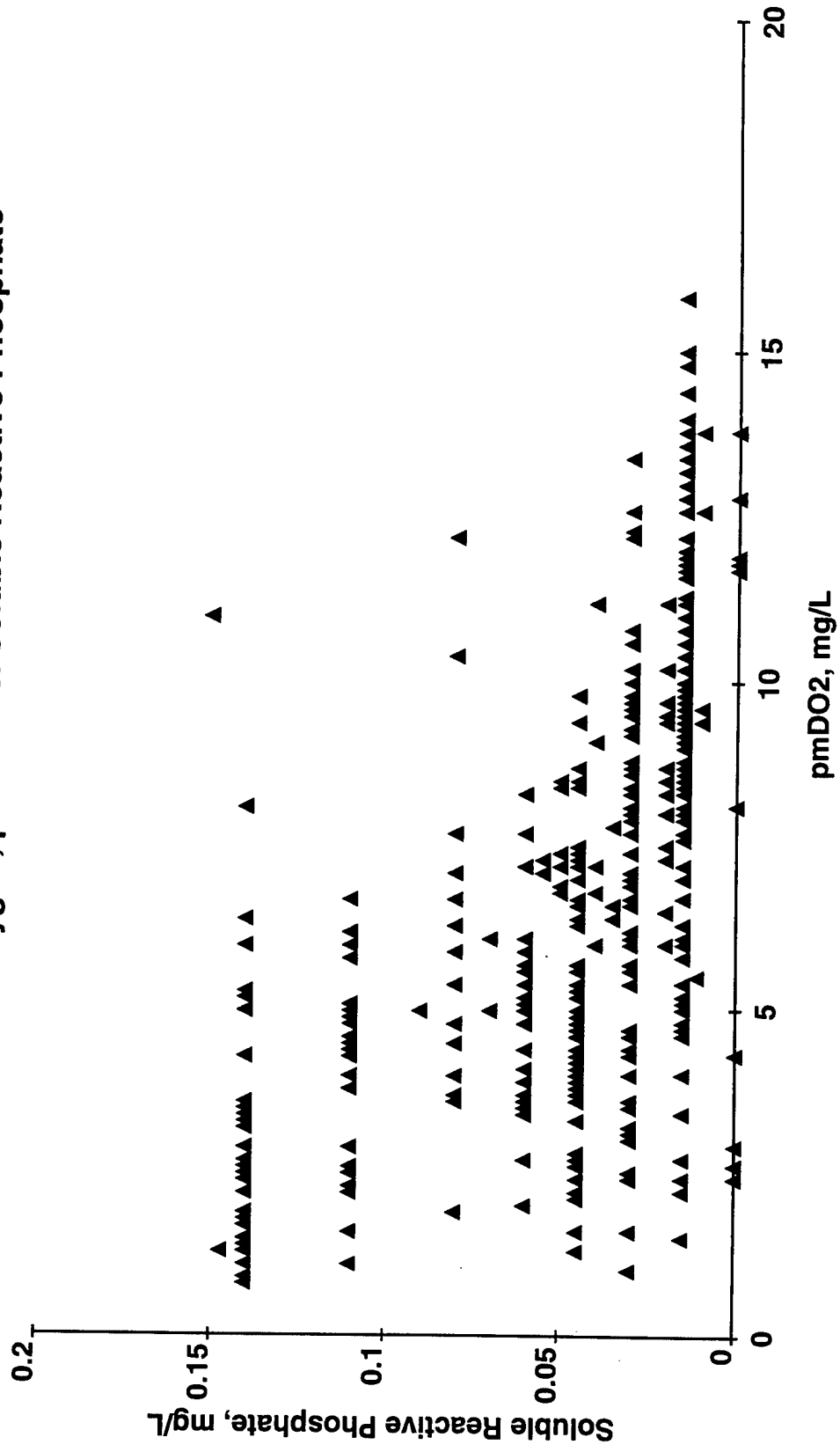
Figure 108. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, pmDO2 vs. TTC Respiration
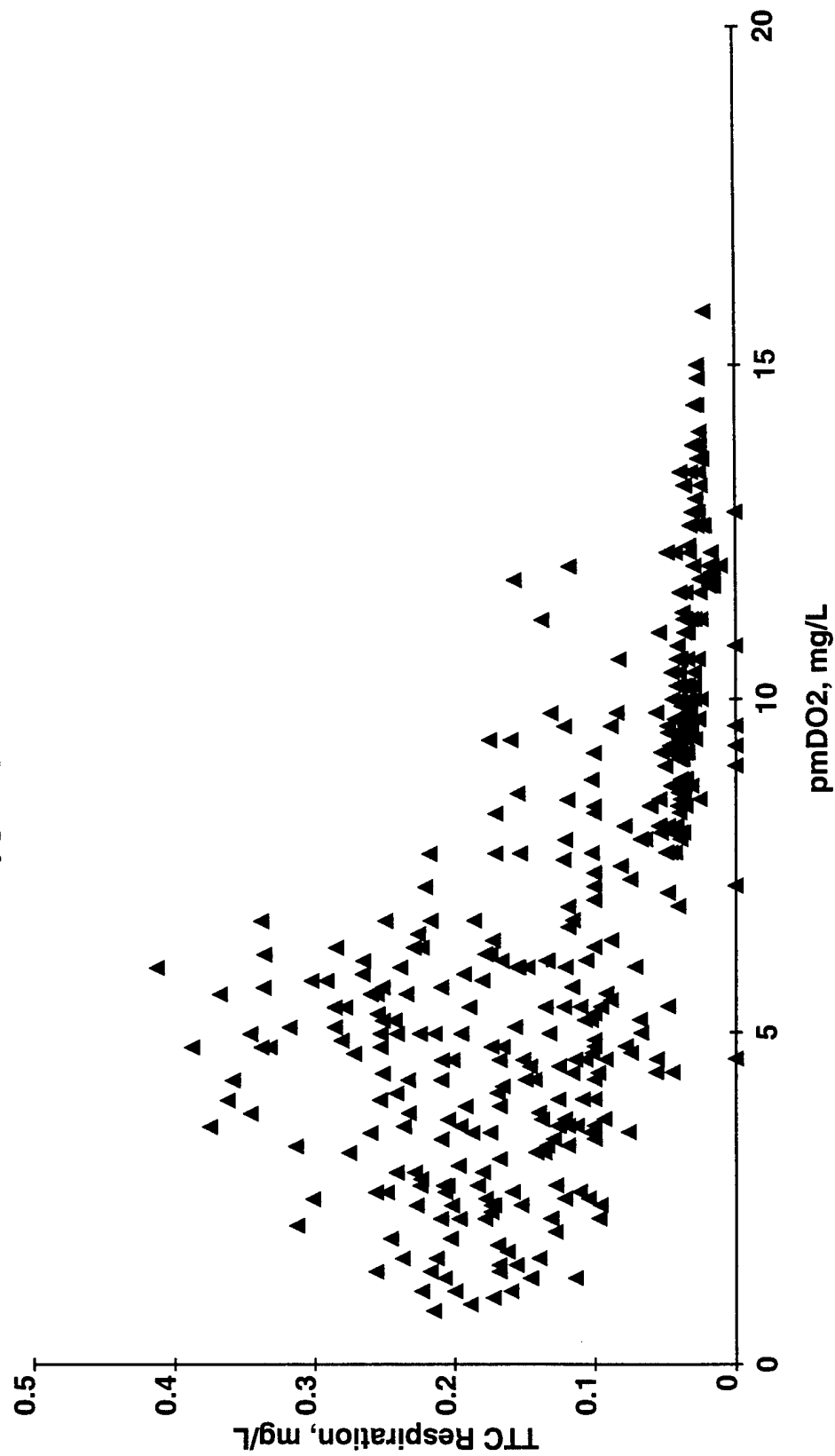
Figure 109. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO3 vs. Soluble Reactive Phosphate
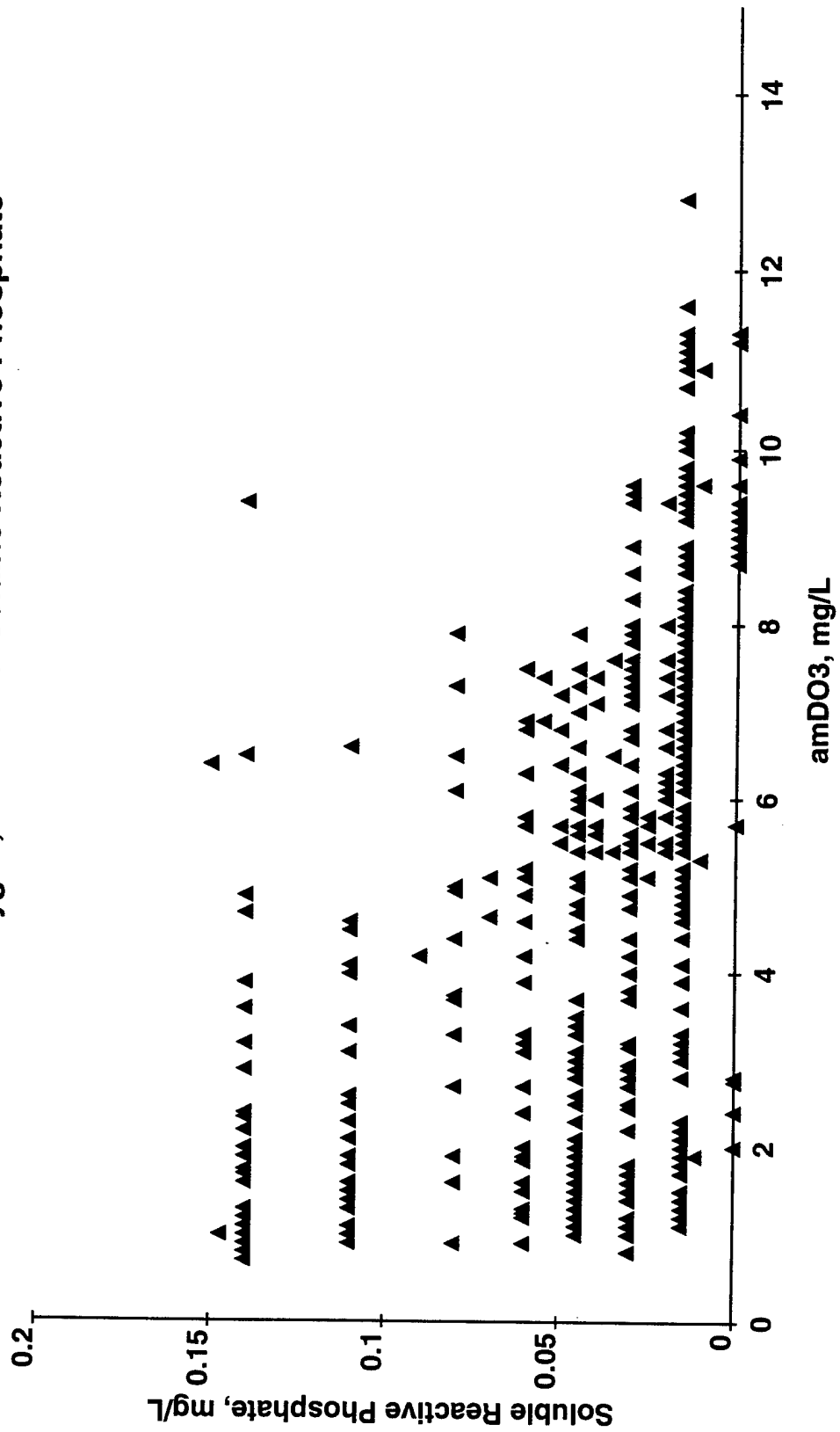
Figure 110. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO3 vs. TTC Respiration
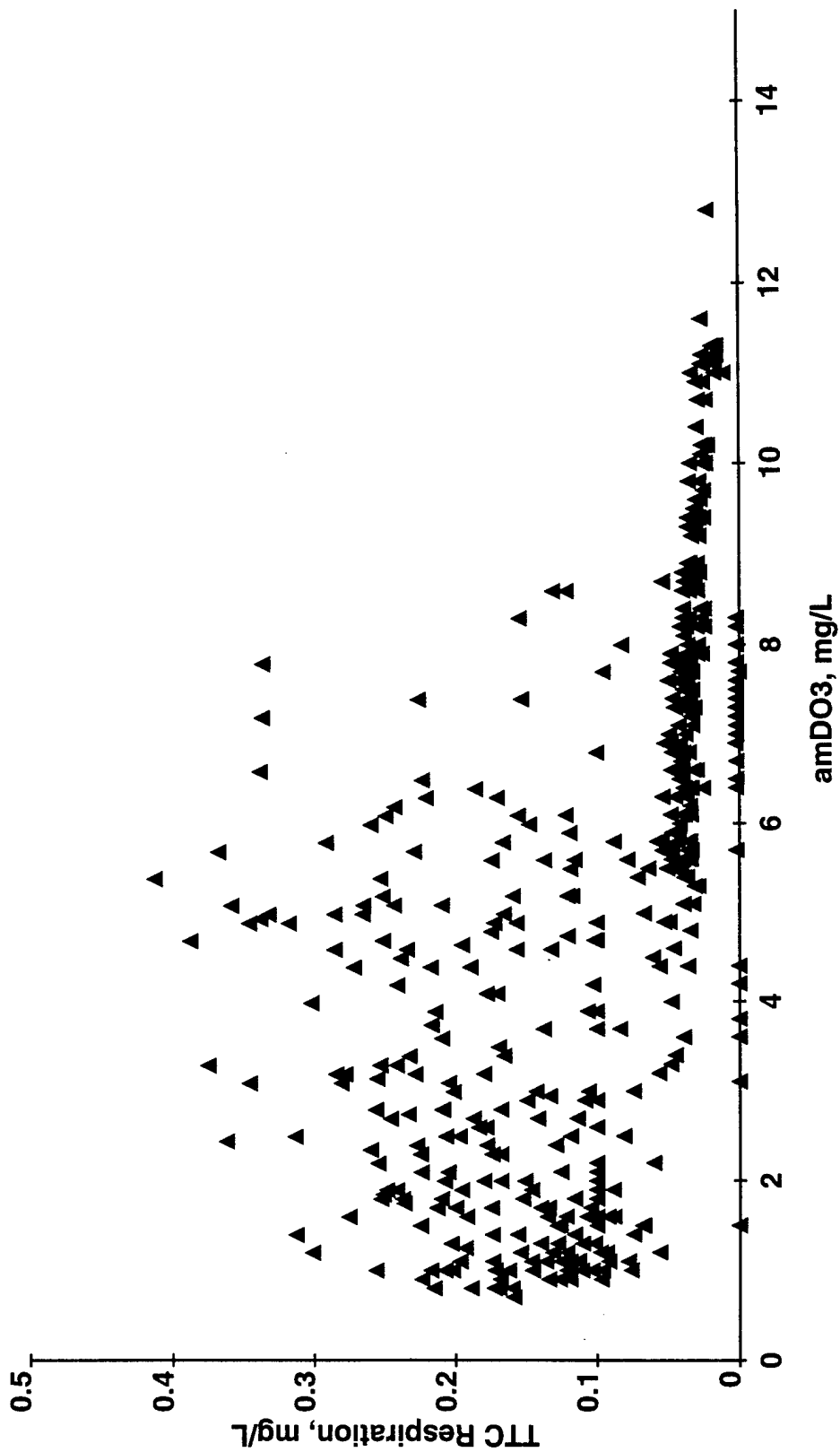
Figure 111. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO3 vs. Ankistrodesmus
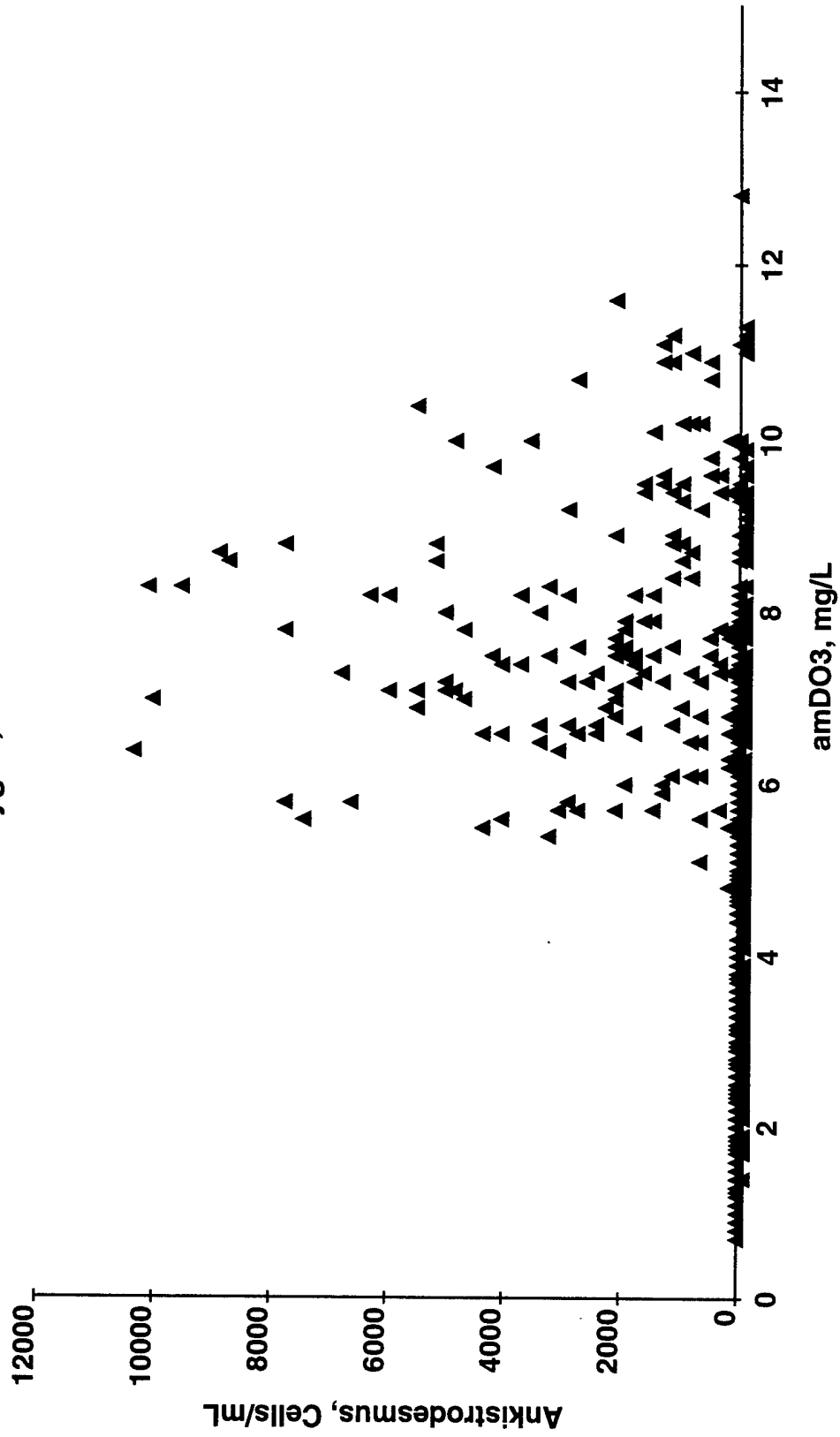
Figure 112. Phytoplankton and Water Quality Correlations
Dissolved Oxygen, amDO3 vs. Pennate Diatoms
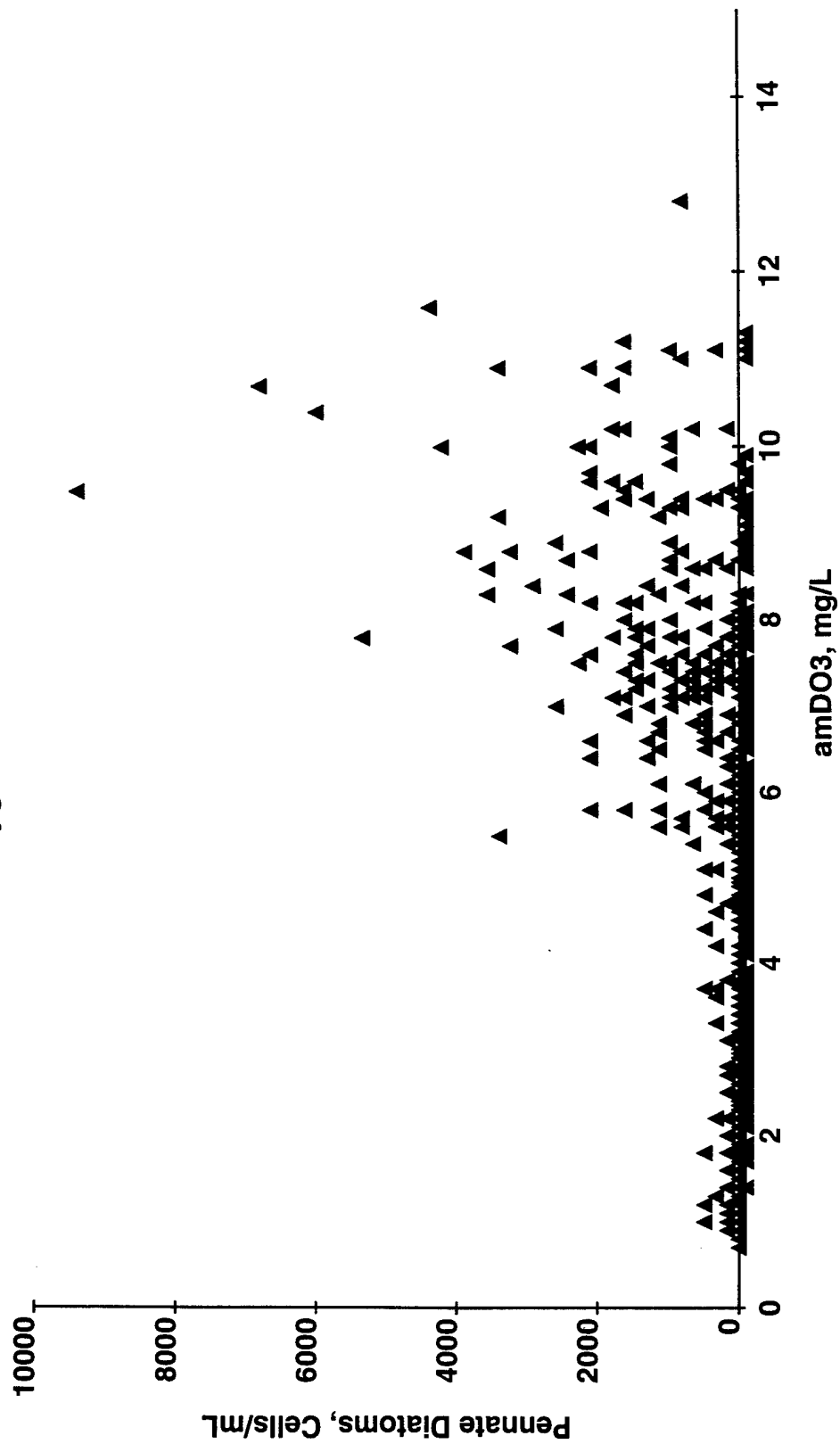
Figure 113. Phytoplankton and Water Quality Correlations
Heterotrophic Flagellates vs. Ciliates
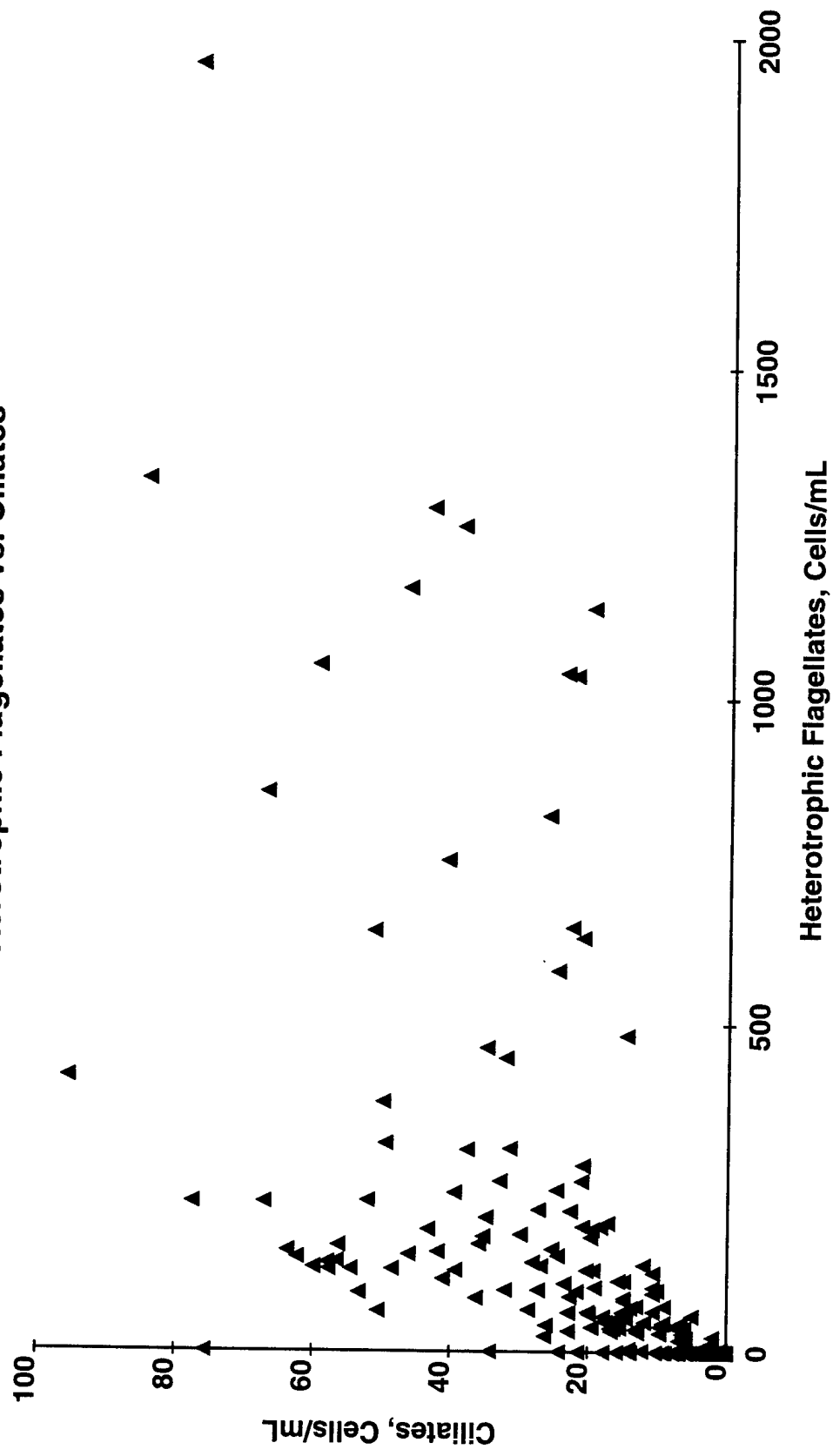
Figure 114. Phytoplankton and Water Quality Correlations
Pennate Diatoms vs. TTC Respiration
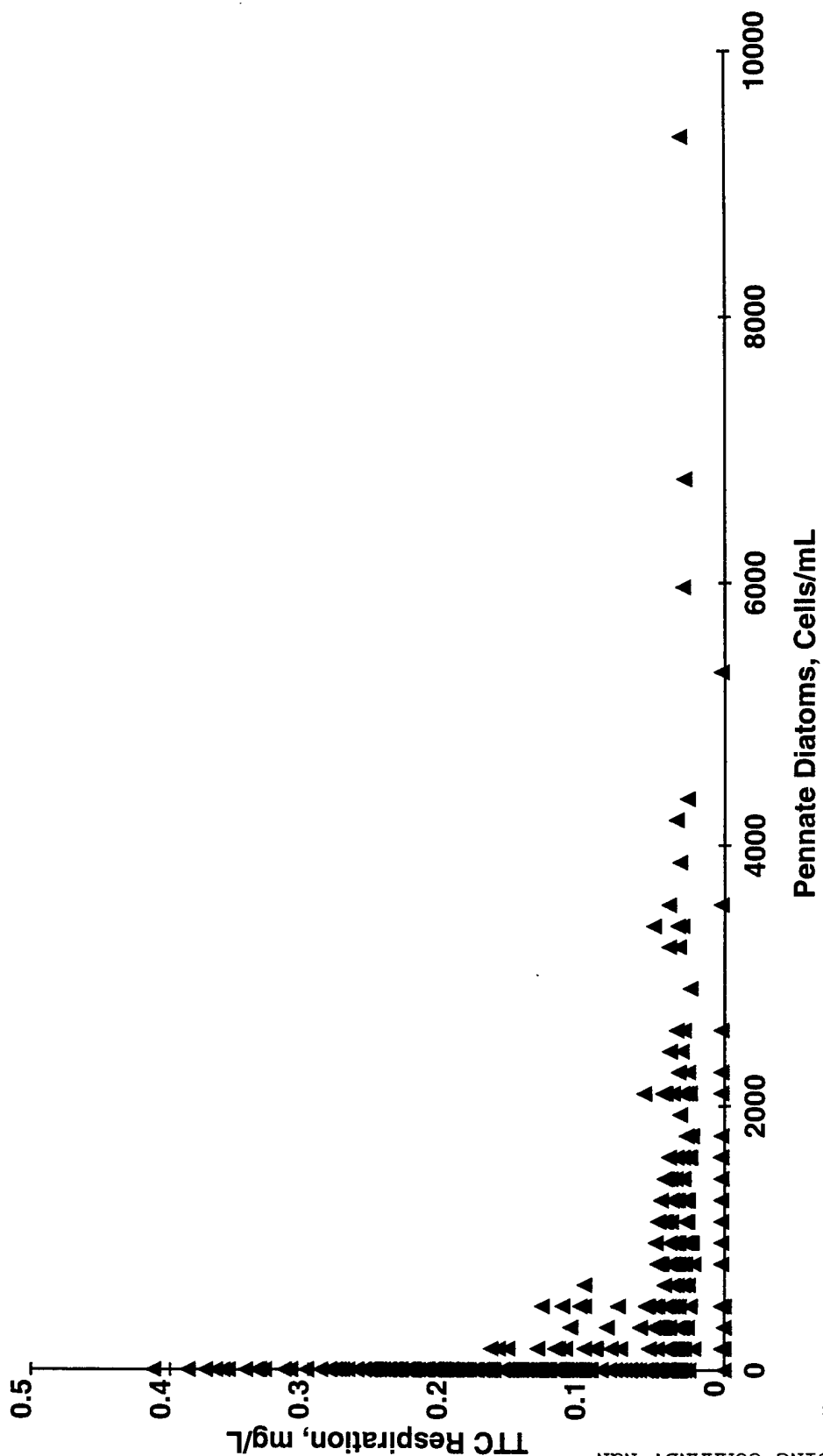
Figure 115. Phytoplankton and Water Quality Correlations
pH vs. Dissolved Oxygen, amDO1
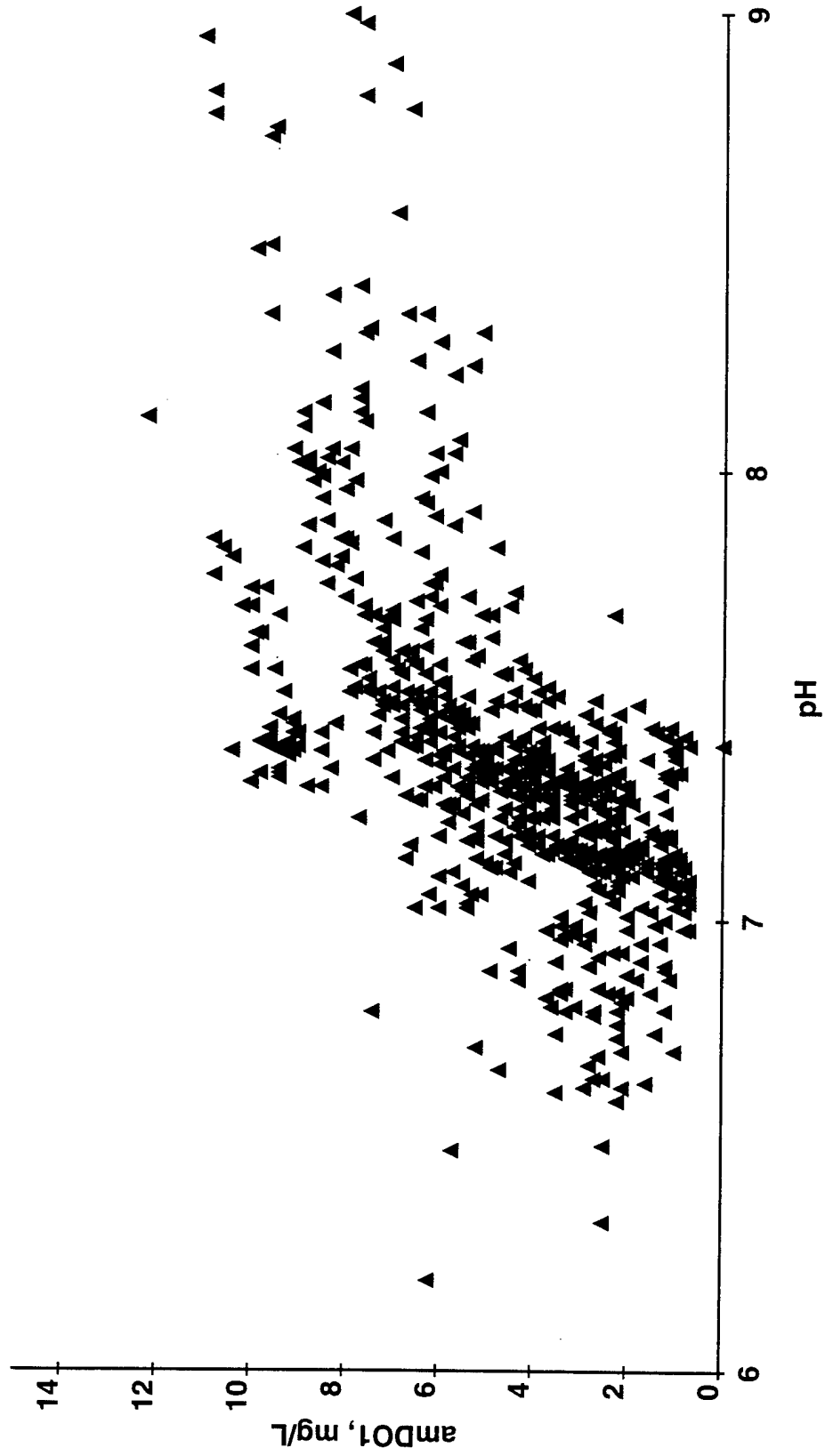
Figure 116. Phytoplankton and Water Quality Correlations
pH vs. Dissolved Oxygen, pmDO2
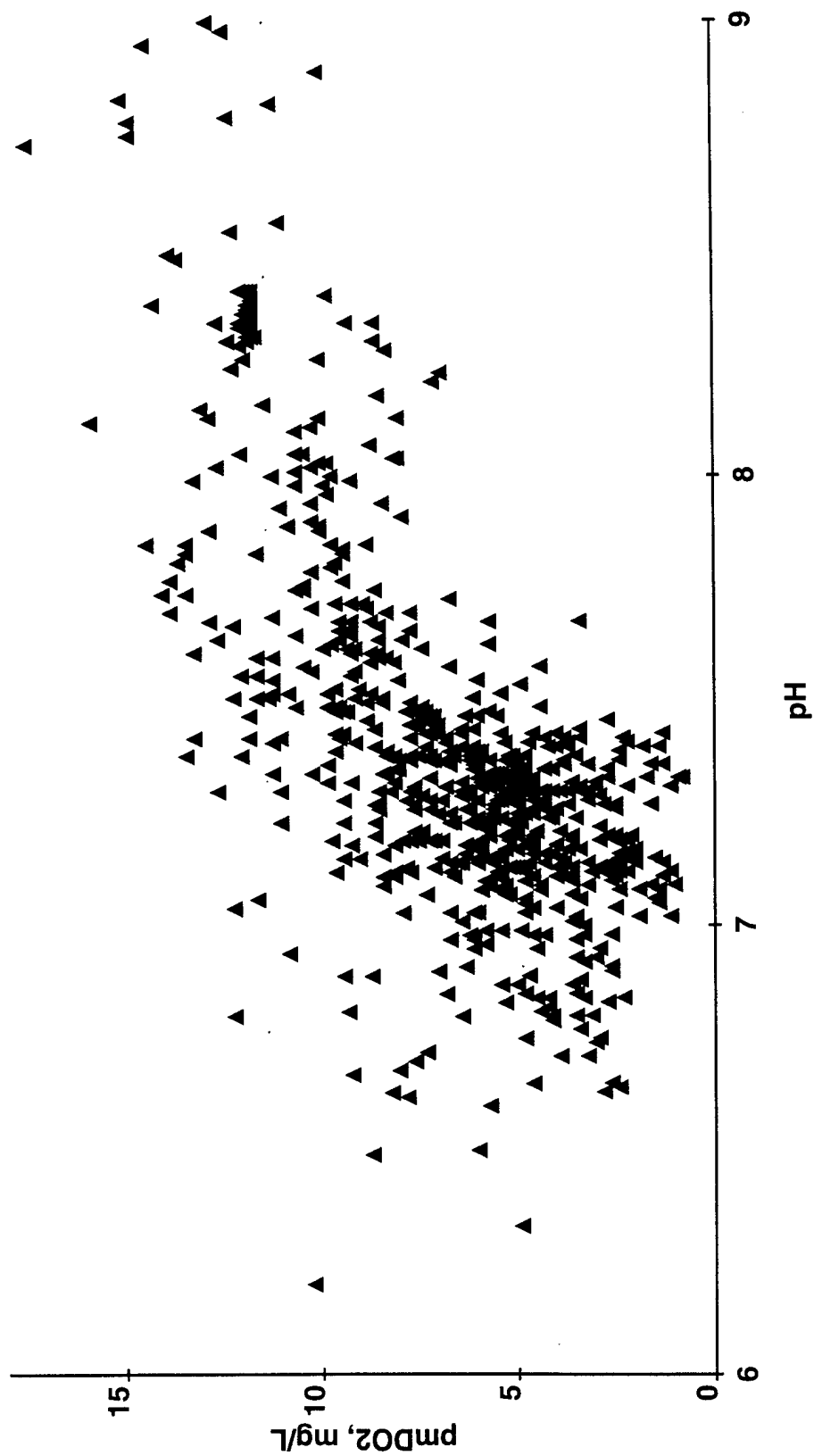
Figure 117. Phytoplankton and Water Quality Correlations
pH vs. Dissolved Oxygen, amDO3
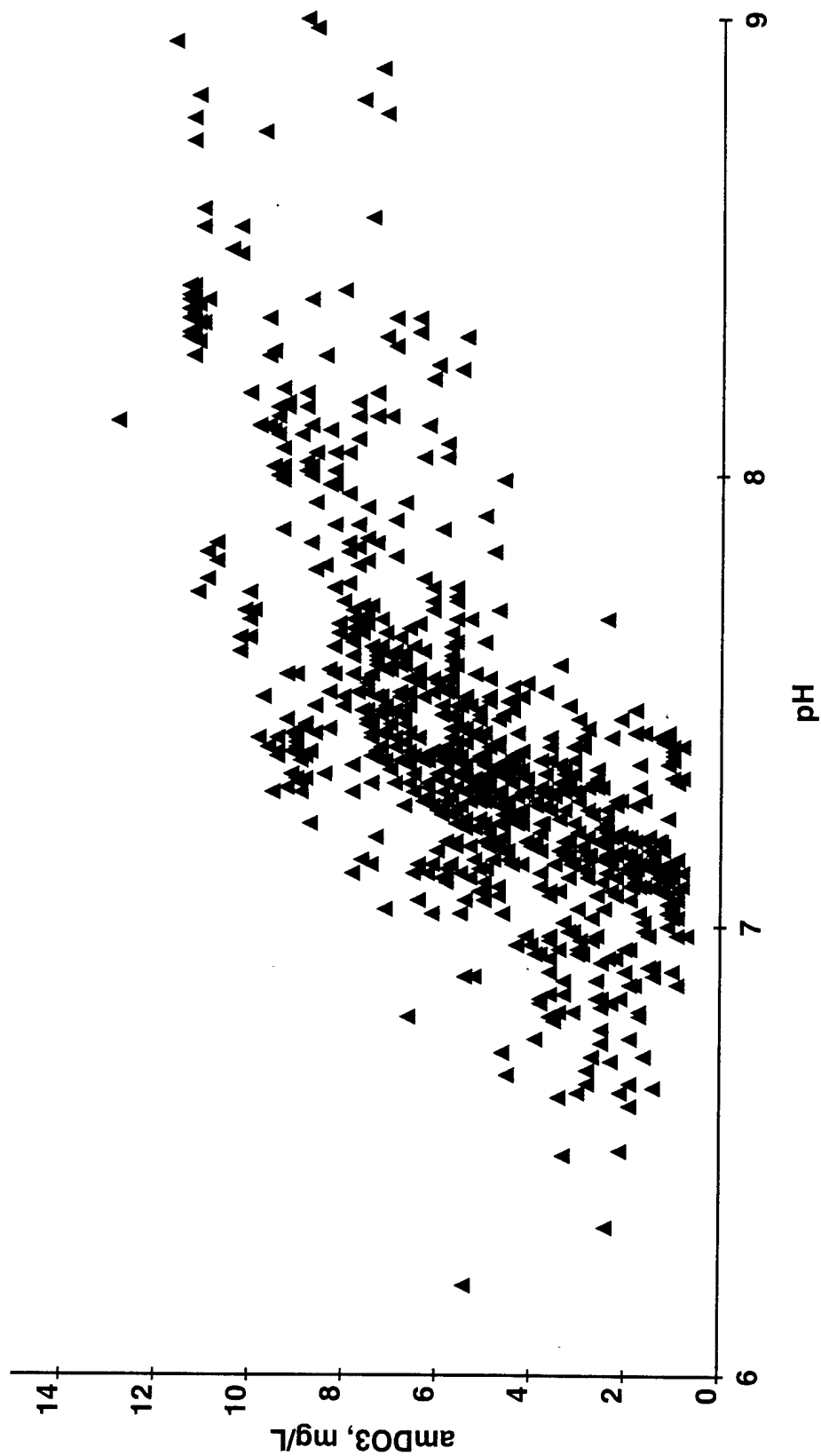
Figure 118. Phytoplankton and Water Quality Correlations
pH vs. Pennate Diatoms
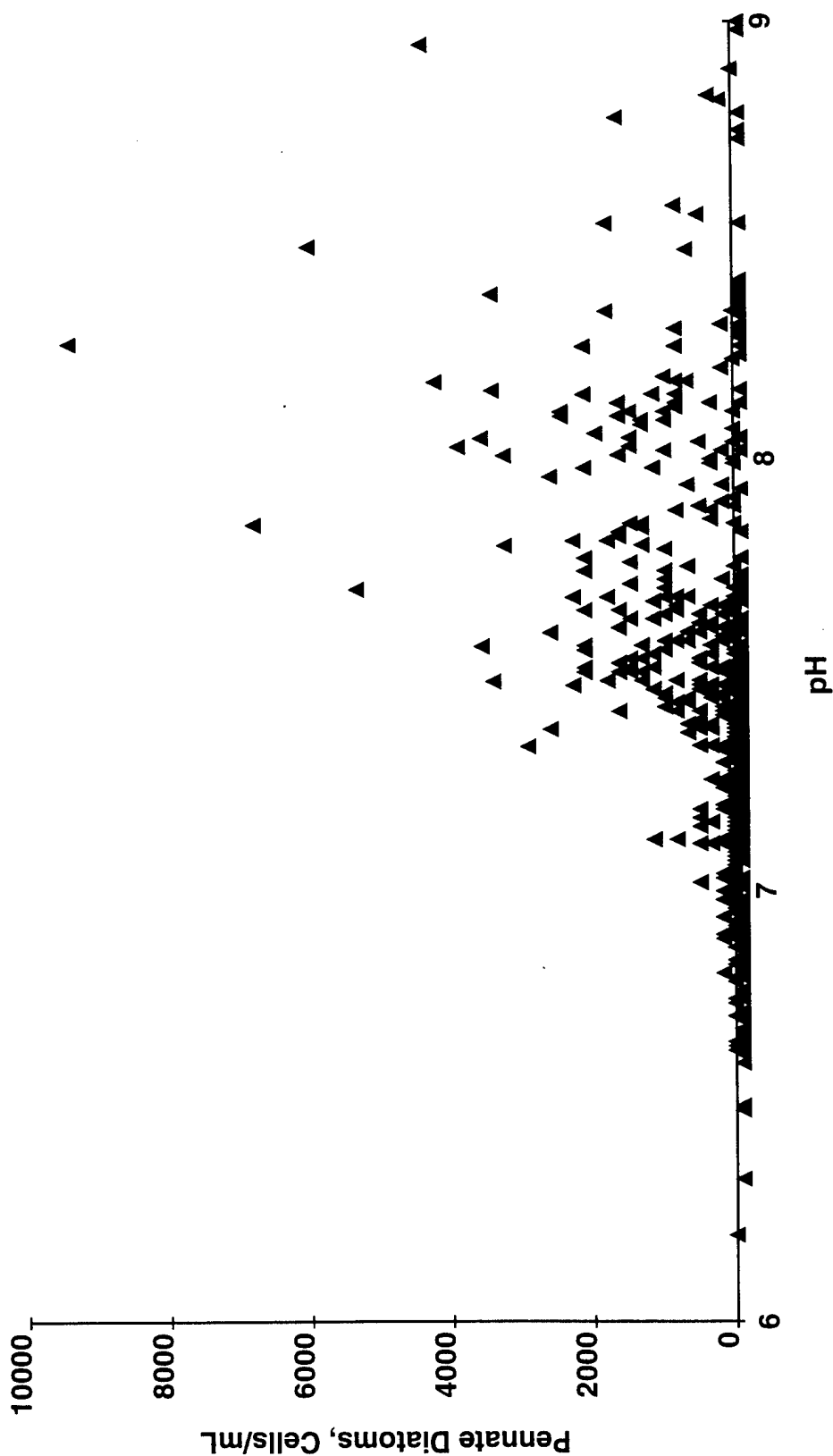
Figure 119. Phytoplankton and Water Quality Correlations
pH vs. TTC Respiration
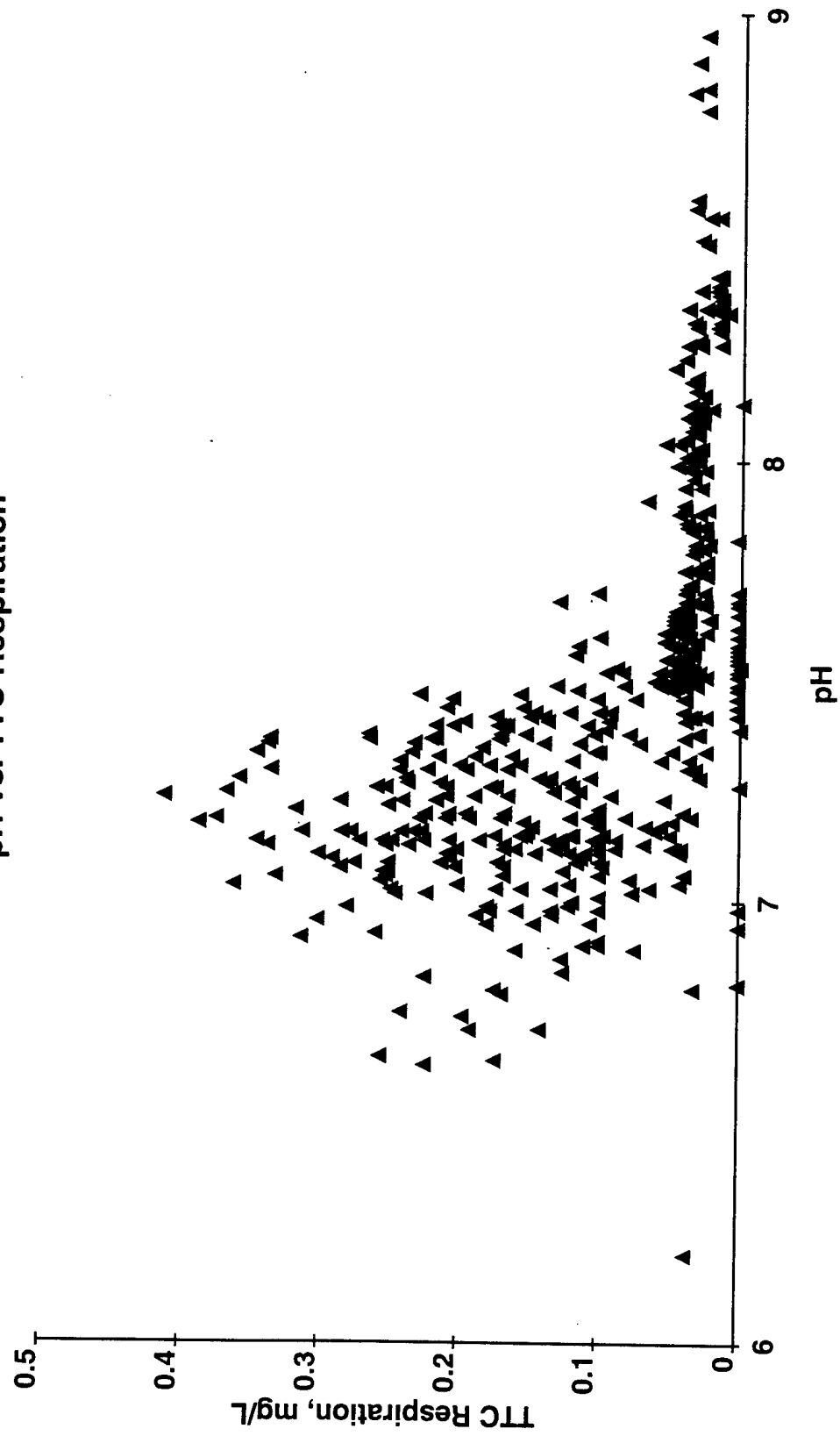
Figure 120. Phytoplankton and Water Quality Correlations
Schroederia vs. Pennate Diatoms
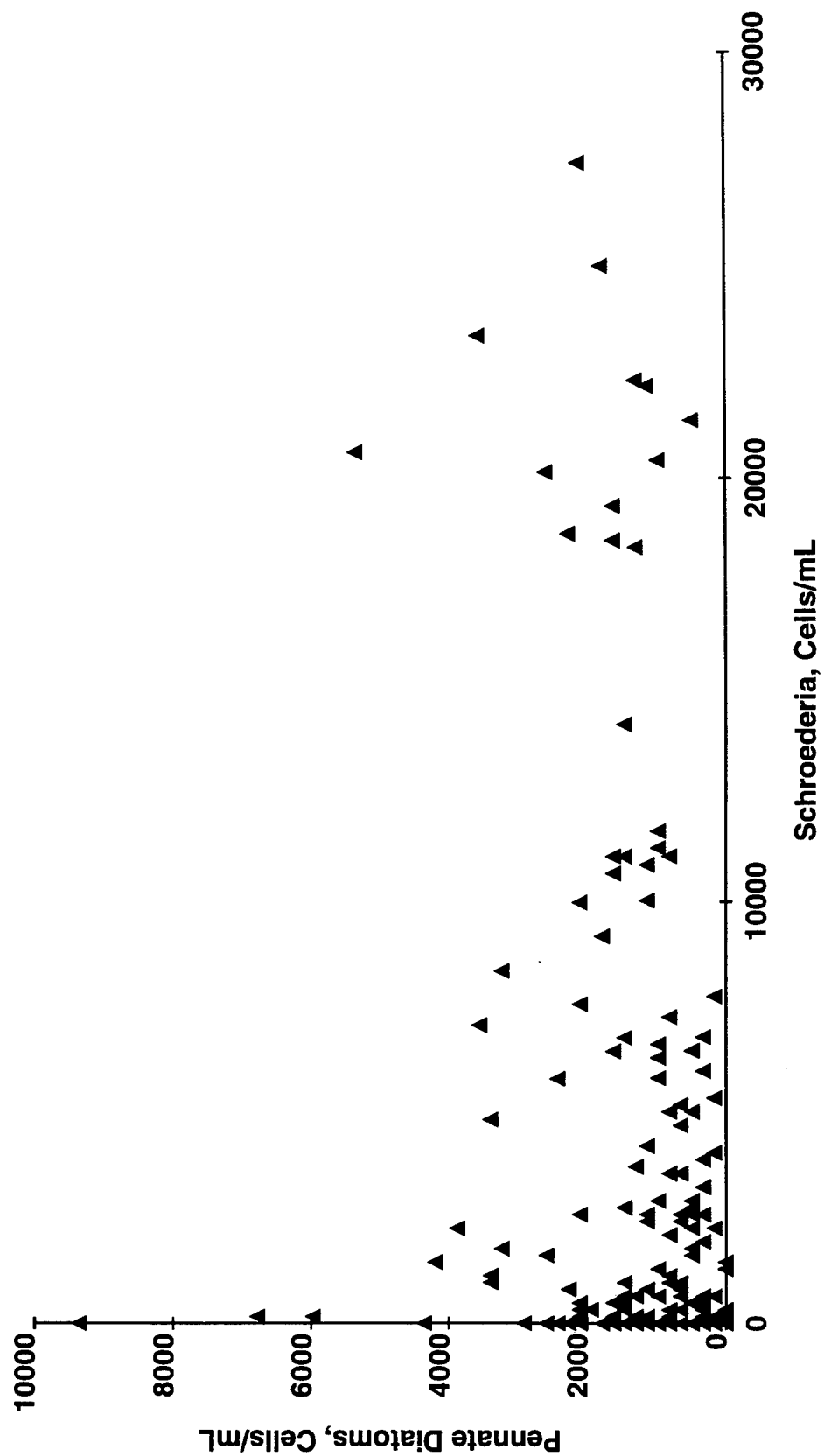
Figure 121. Phytoplankton and Water Quality Correlations
Schroederia vs. Heterotrophic Flagellates
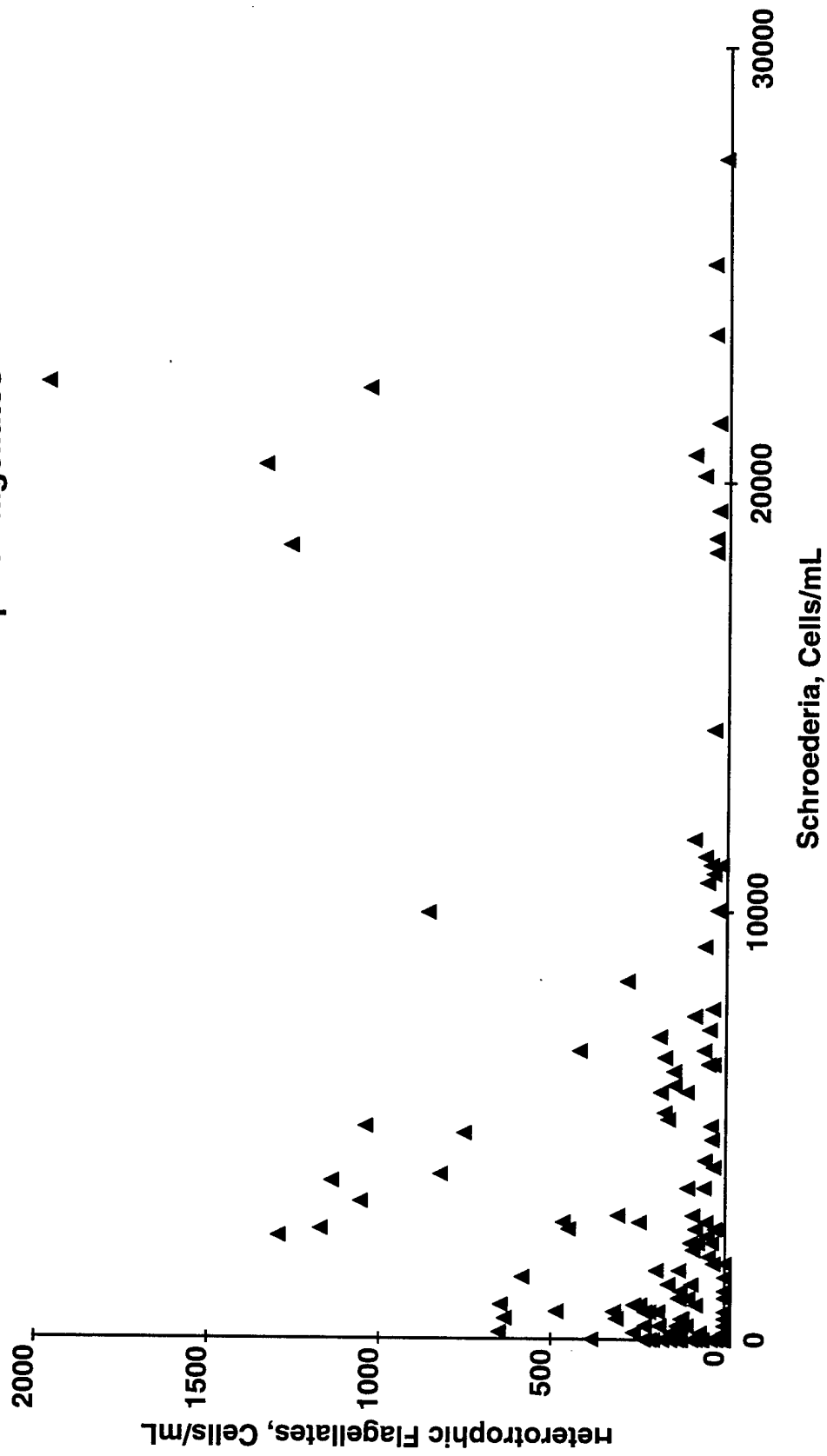
Figure 122. Phytoplankton and Water Quality Correlations
Total Solids vs. Total Volatile Solids
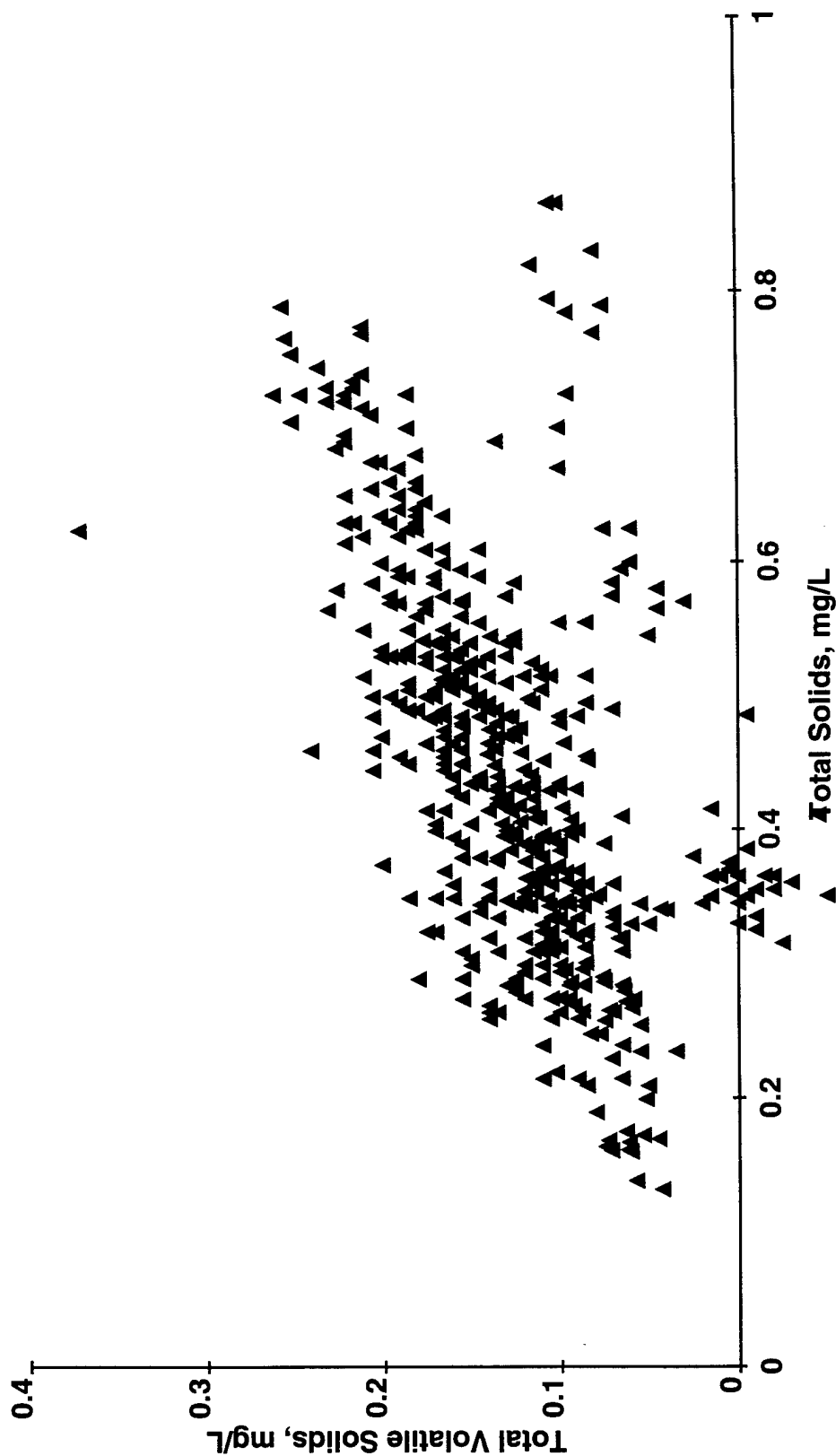
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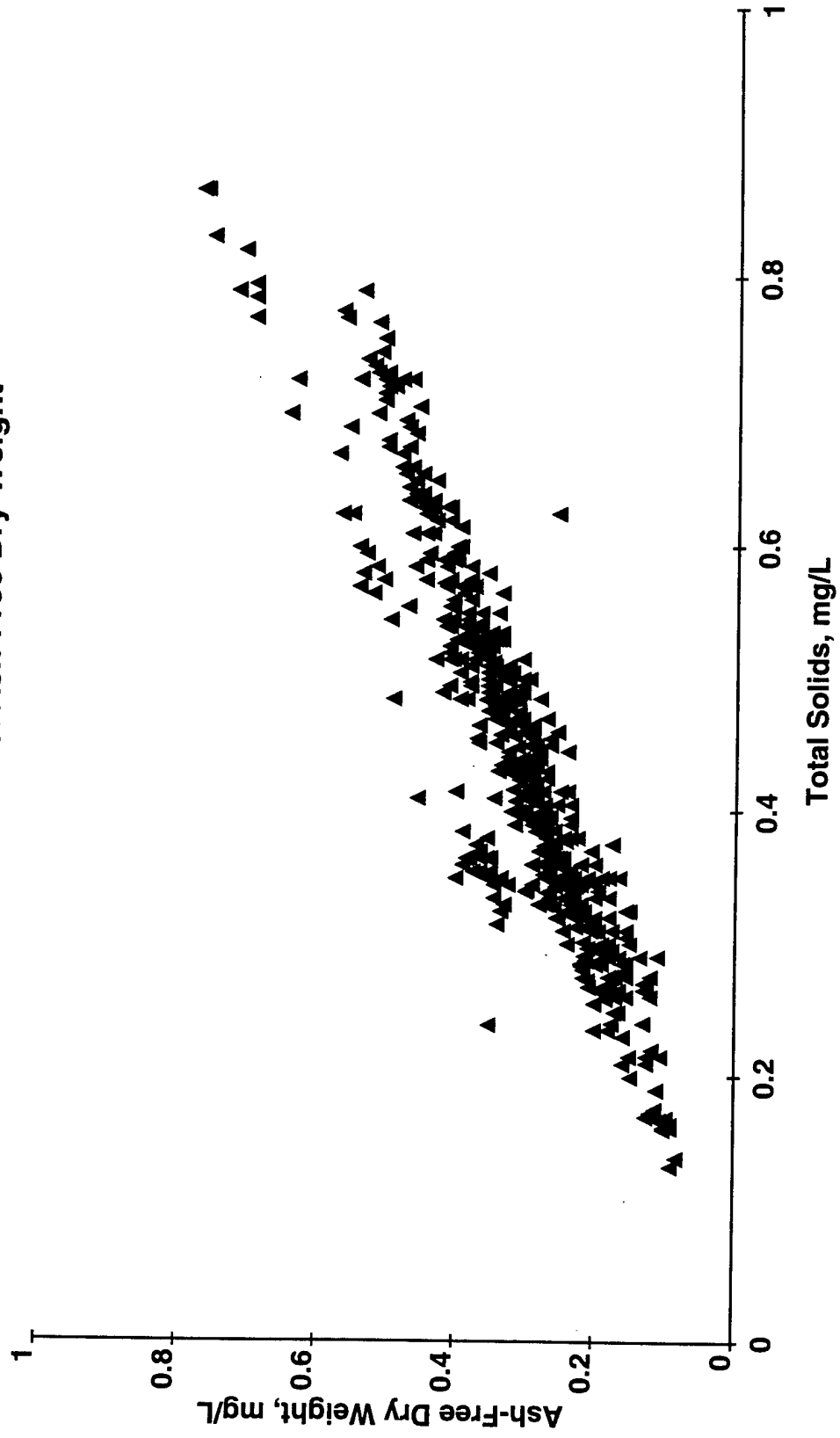
Figure 124. Riffle Cluster Quality
Periphyton and Leaf Pack Data
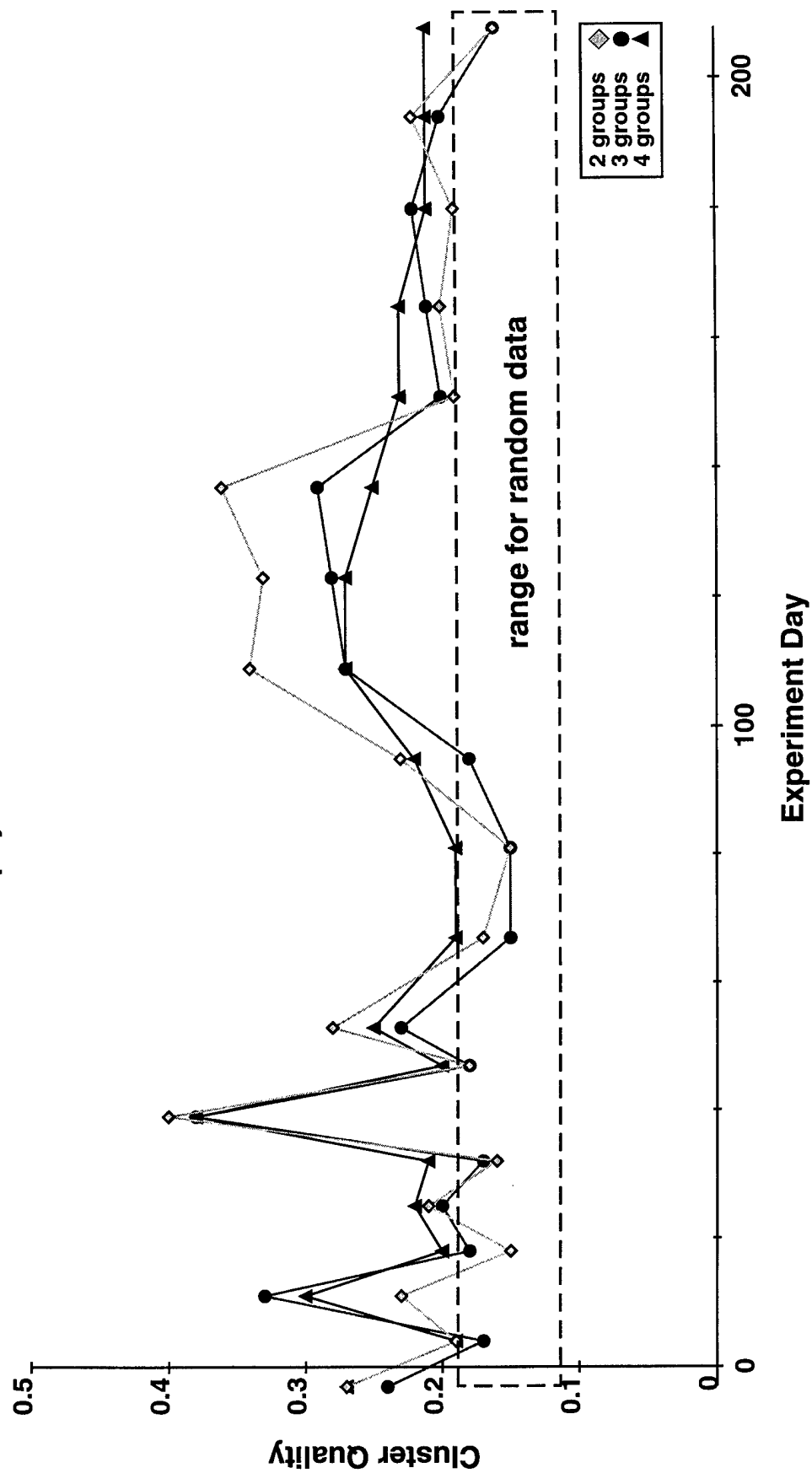
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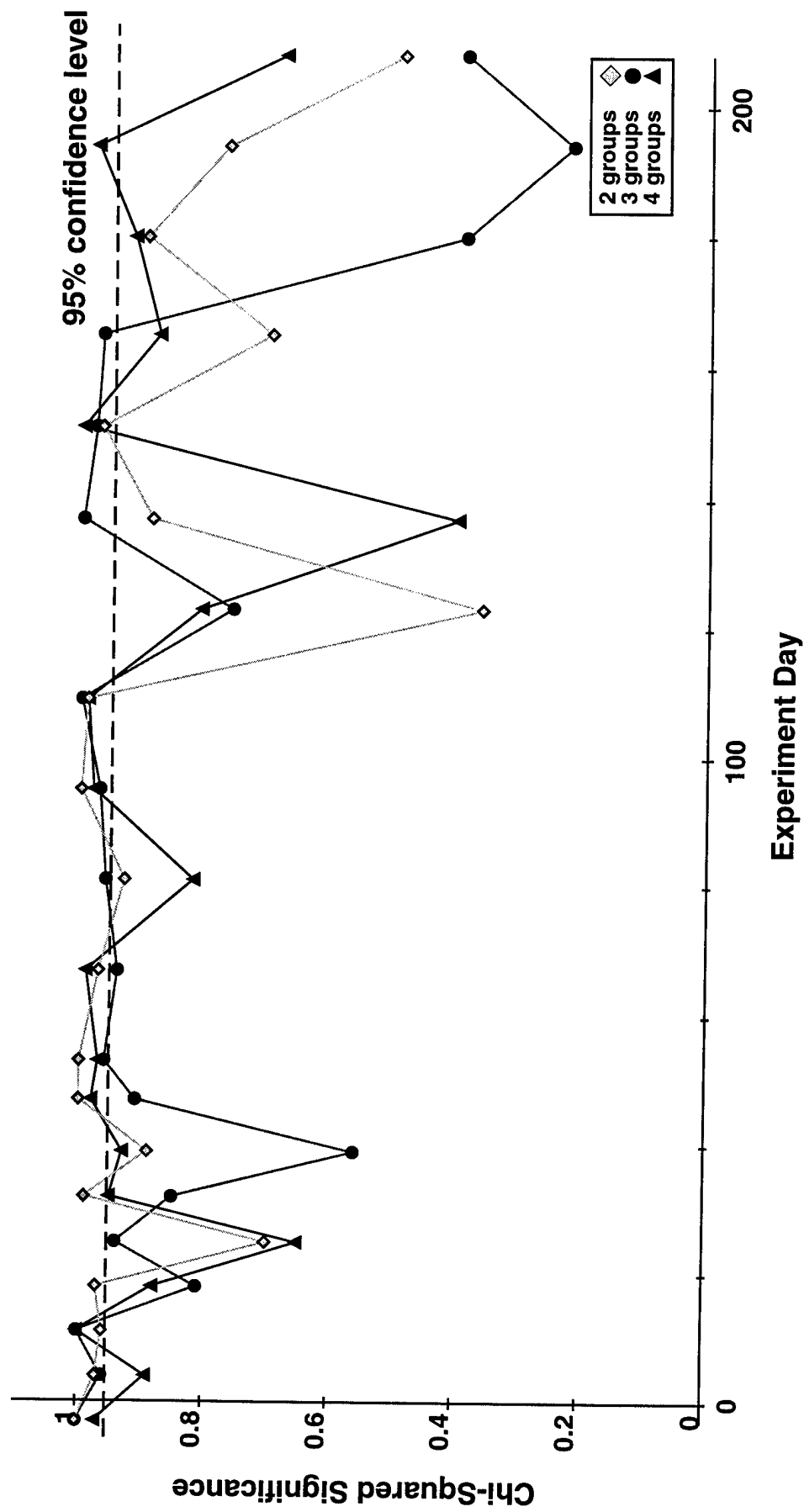
Figure 126. Riffle Cluster Quality
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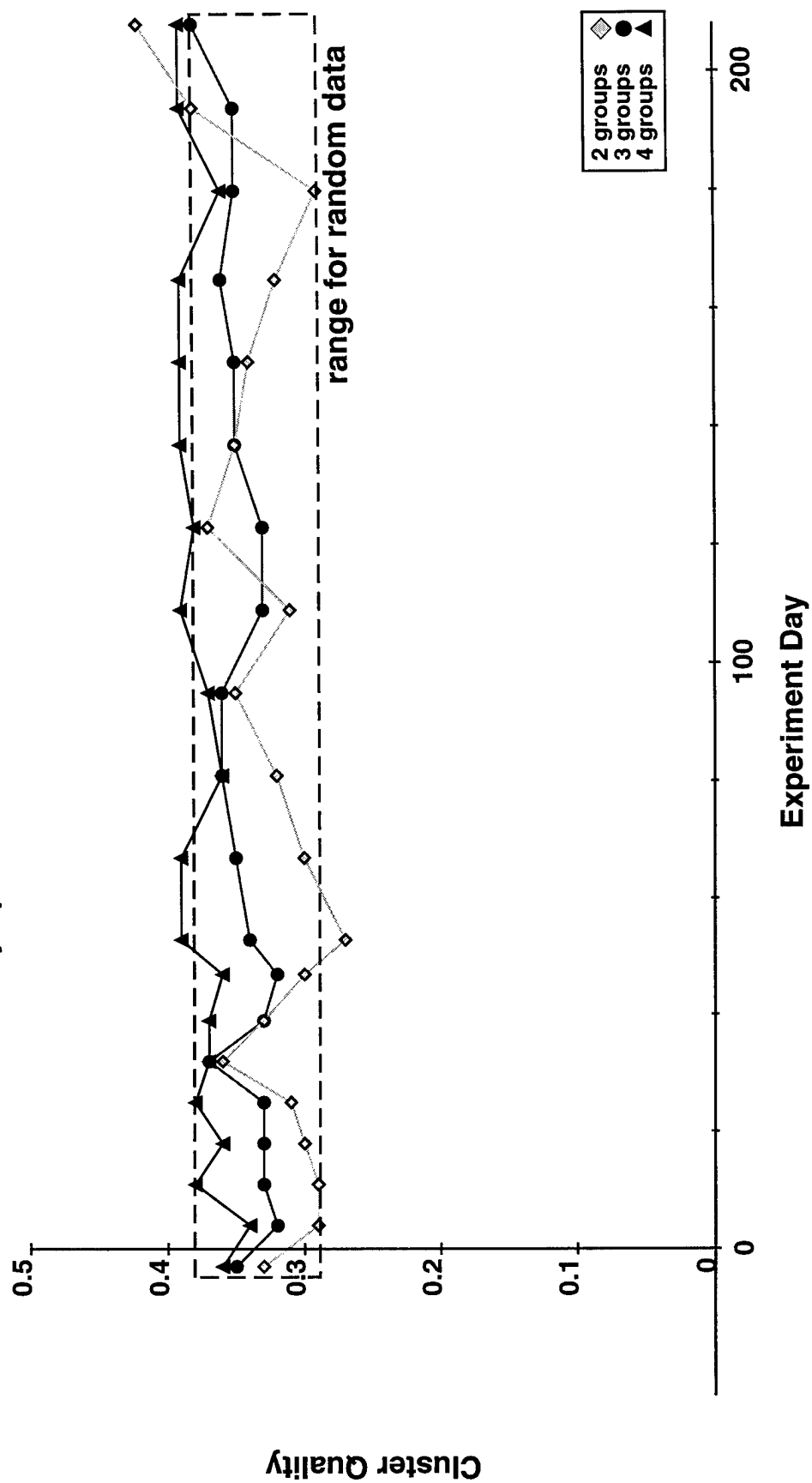
Figure 127. Association Analysis Results
Phytoplankton and Water Quality Data
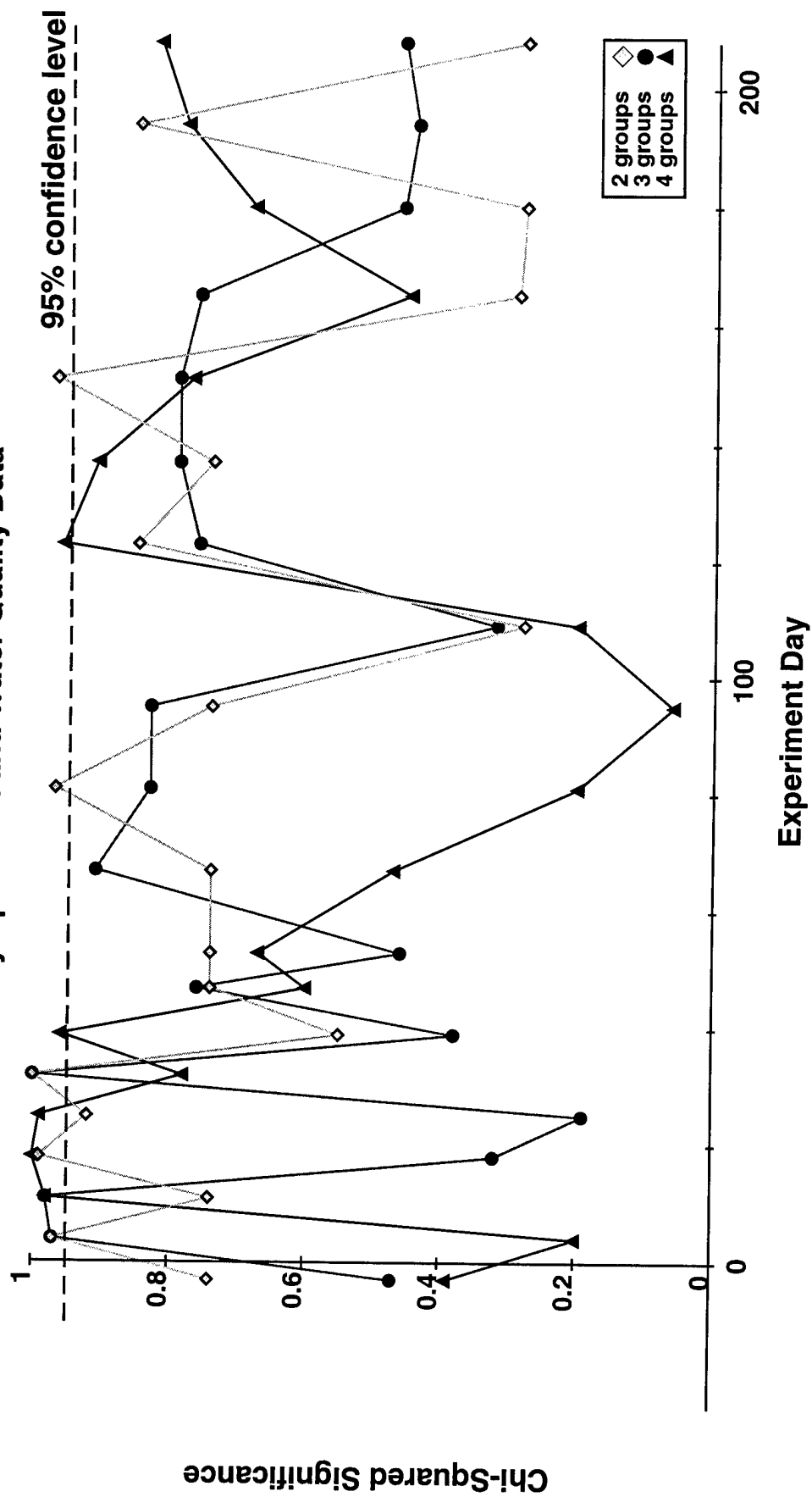
Figure 128. Riffle Cluster Quality
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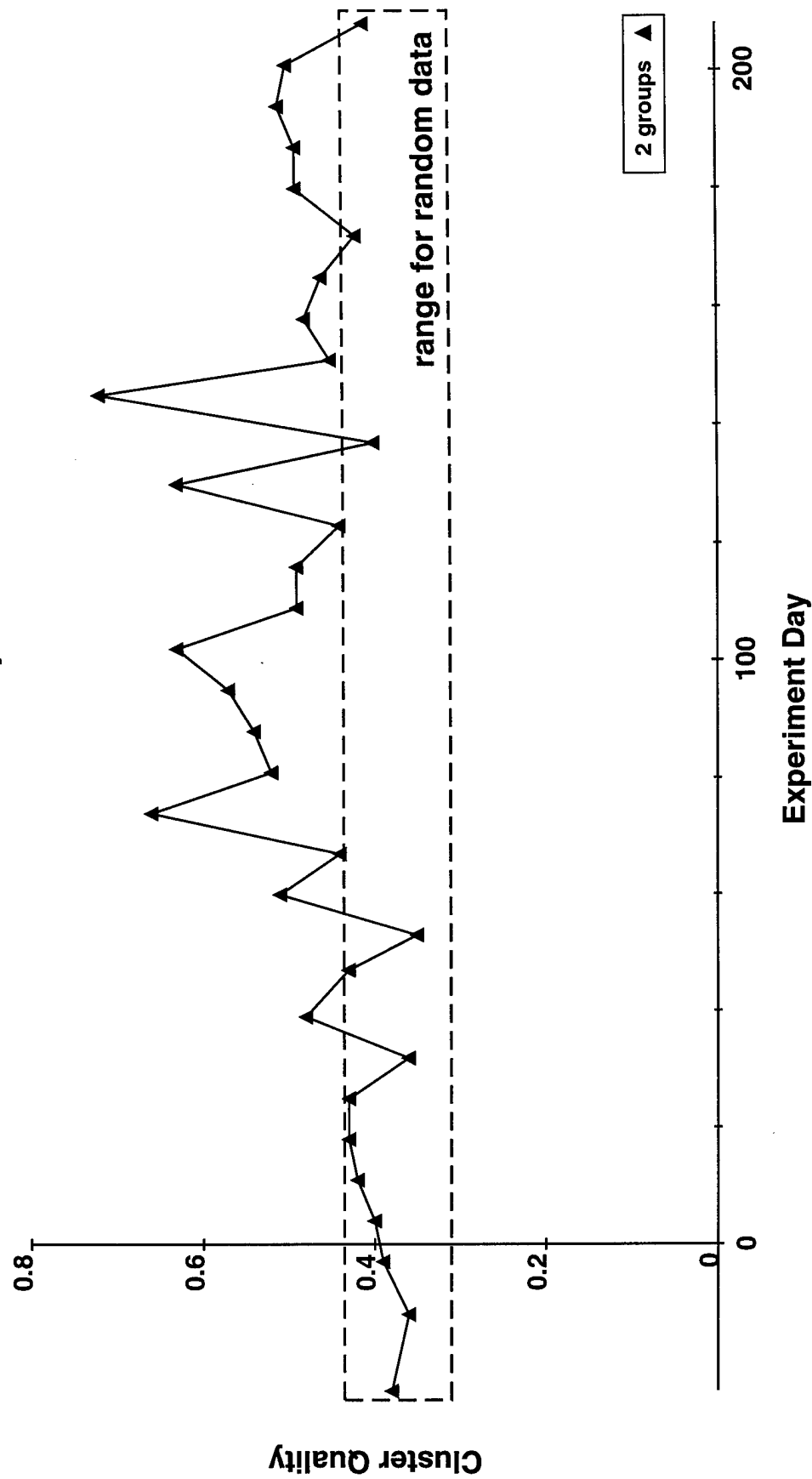
Figure 129. Association Analysis Results
Water Quality Data
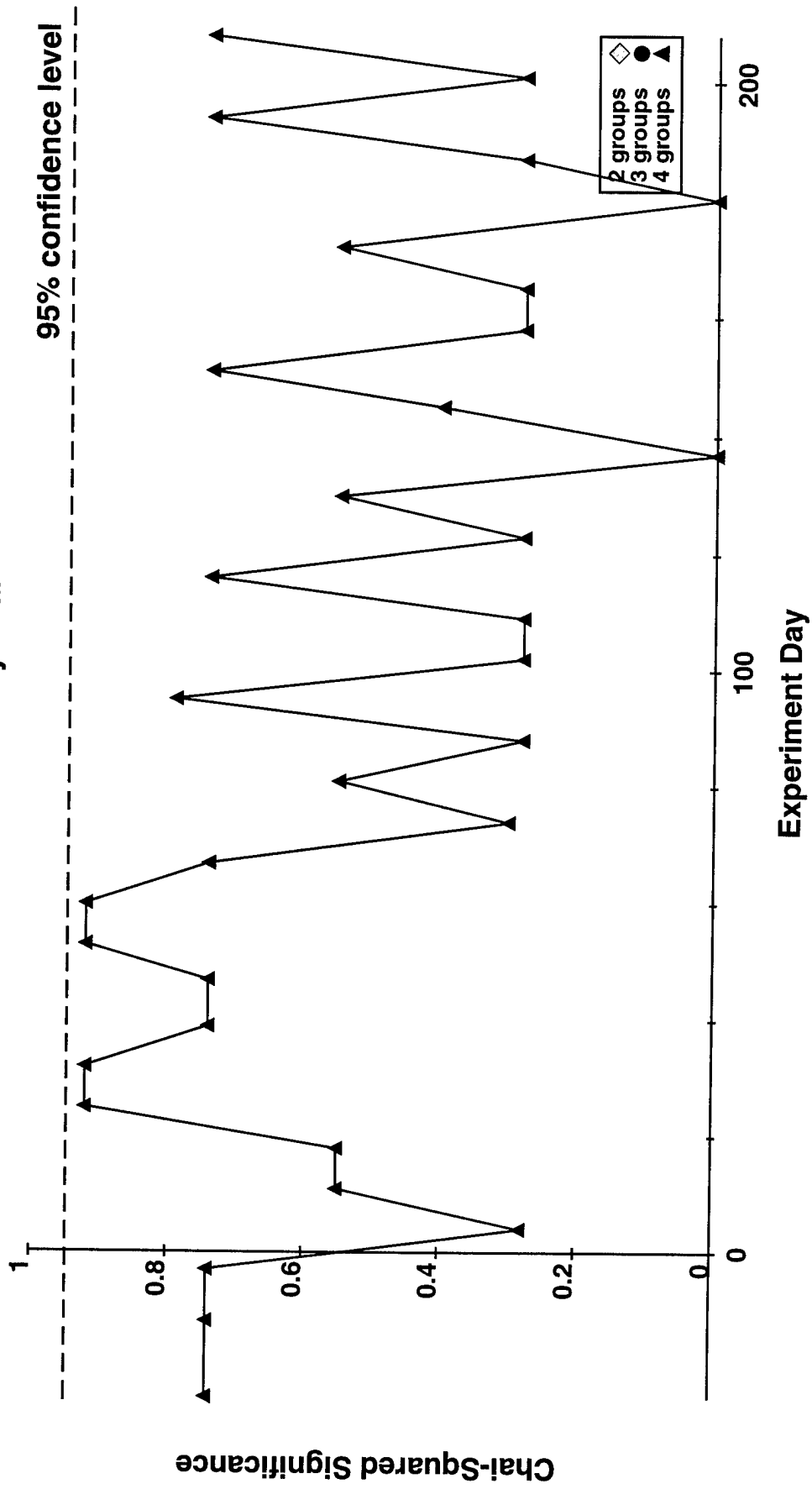
Section 4: Multiple Stressor JP-8/Heat and JP-8/JP-8 Experiments

Introduction

One of the primary issues in ecotoxicology today involves the long term impacts of disturbances on natural ecological systems. The conventional ecological paradigm supports stability and recovery and finds its basis in the idea that a given environment has an ideal state (Clements 1916). Under this paradigm, systems will return to their original, stable state following a disturbance indicating that the system is reversible. The return of a system to this original state such that it responds to a subsequent disturbance as if the first perturbation had not occurred is defined as stability. Recovery is the process that returns the disturbed system to the original state such that it responds to a subsequent disturbance as if the first perturbation had not occurred (Landis et al. 1996)

One of the main problems with stability-recovery theory lies with the application of population stability theory to natural communities without first checking to see that stability is a valid assumption in nature (Connell and Sousa 1983). In a search for evidence to support the ideas of stability and recovery, Connell and Sousa conducted an extensive search of the ecological literature but found no evidence supporting stability beyond one turnover of the populations or communities of interest. This not only questions stability and recovery as properties of natural ecological systems, but also supports Elton's (1930) argument against a balance of nature. Thus, stability and recovery may not be appropriate models for ecological systems. Consequently, Connell and Sousa (1983) proposed persistence within bounds as a better description of what is seen in nature.

Reice (1994) has proposed non-equilibrium ecology as an explanation for the lack of detectable stability in natural systems. Under this view, systems are always in a state of change, continually "recovering" from disturbances. Recovery to an original state, and, hence stability, is not seen is because a system is disturbed again before it can reach the recovered state. While non-equilibrium ecology as described by Reice provides an explanation for the lack of stability in nature, the concept maintains that ecological systems still have the ability to return to an original state or rather, that ecological systems are reversible.

However, there are those suggesting that ecological systems lack stability because they maintain a memory of disturbances and are changed irreversibly (Landis et al. *in press[b]*, 1993, 1994, *in press[a]*). Drake (1991) demonstrated the importance of history in determining the response of a system to a disturbance. In his microcosm study, differences in the timing of the inoculation of several different algal species produced very different responses to the invasion of a consumer species. In a second experiment using microcosms as islands with differing invasion schemes, Drake et al. (1993) again found that different histories (timing, invasion success, and persistence of organisms) made for systems that responded differently to a stressor event.

In addition to the historical nature of ecological systems, Brooks (1989) has commented on their irreversibility and evidence of this property has been found by Landis et al. (1993, 1994) in microcosm studies. In these studies, treatments demonstrated a dose related divergence from the reference group followed by an apparent convergence. However, another divergence followed such that the treatments were distinguishable from one another. Consequently, what appeared to be recovery was nothing more than a confluence in time. The systems "remembered" which treatment they belonged to and responded accordingly.

Community Conditioning Hypothesis

The viewpoint that argues against recovery has been clarified in the form of the community conditioning hypothesis as stated by Matthews, Landis, and Matthews (1996) ecological communities retain information about all events in their history. This suggests that communities are a product of their unique etiology because they tend to preserve the information imparted to them by all of the biological, physical, and chemical events in their history. It logically follows that no two ecological systems will ever be the same. Changes in community dynamics,

therefore, are attributable to the history of the community, specifically, the conditioning of the treated community by stress events. Consequently, systems exposed to different stressors would be expected to exhibit different dynamics (see also Landis et al. *in press[b]*).

Community conditioning strongly questions the traditional ideas of recovery and stability. However, because a system may maintain the information imparted by a disturbance without showing any change for long time intervals, it becomes difficult to demonstrate that conditioning has occurred. Yet, just because there is no detected effect does not mean there is no effect. As past microcosm studies have demonstrated, even though treated communities appear to be very similar at a point in time, they maintain a separate identity that appears later in the test period (Landis et al. 1993a, 1994). At this point of confluence, the information that dictates change later in the system is present but stored in an uncataloged array of parameters (Landis et al. *in press[b]*). Consequently, the ability to predict the trajectory of the systems is limited by the ability to detect changes.

Types of Effects

The changes that give ecological systems a unique identity may be readily apparent or may remain hidden for long periods of time. These changes, as discussed by Landis and Yu (1995), may be direct or indirect effects. Toxicologically speaking, direct effects are those caused by the molecular interaction of the toxicant and the receptor site. Direct effects occur at the level of the individual and include inhibition of an enzyme system, behavioral alterations, mutagenesis, and oncogenesis.

Indirect effects are those that are not mediated by direct interaction of the toxicant and the receptor site. Indirect effects include things such as alterations in predator-prey interactions, changes in nutrient cycling, and alterations in the detrital community. Indirect effects occur at all levels of biological organization from the individual to the community or ecological system (Landis and Yu 1995).

Indirect effects have received relatively little attention even though they are very much a part of disturbances. Indirect effects are initiated with direct effects but may be masked by the direct effects. This can make indirect effects difficult to detect. The changes they cause in the system are present, nonetheless. Indirect effects may also appear long after the stressor has left the system. These lingering effects have been shown in many microcosm studies (Landis et al. 1993a, 1994; Drake 1991; Drake et al. 1993). Indirect effects certainly play an integral role in defining the change to an ecological system and are an important part of community conditioning.

Microcosms as Models

Microcosm studies provide a means to evaluate changes in community dynamics in response to disturbances. Microcosms are designed to model natural ecological systems on a smaller yet trophically similar level. Originally developed to study population dynamics, interactions between species, and community level structural and functional relationships (Beyers 1963; Leffler 1980; Taub 1984), microcosms are now extensively used in chemical fate and toxicological testing (Cairns and Cherry 1993; Landis et al. 1993, 1994). Because microcosm tests are conducted in the confines of the laboratory, a certain degree of control is added to the test. By limiting the natural variances that can occur in natural complex systems, responses to disturbances are more easily detected. Although this compromises the realism of the system, microcosms still provide realism beyond single species testing through their added dimensionality (Cairns and Cherry 1993; Cairns et al. 1992; Giddings 1983).

Microcosms are, however, only physical models of ecological systems. As such, they have the limitations of any model: they are not true or exact representations of natural systems. Microcosms have the properties and components of natural systems but are not miniatures of these systems. Yet, attempts to validate or verify models of ecological systems are often made even though this is simply not possible. Because ecological systems are open, all possible variables cannot be known. This creates data gaps and forces assumptions in model development. When a model does not fit the data, it is very difficult to know why. Conversely, even when a fit is obtained, errors in the model can negate each other making a faulty model

appear correct. Thus, it is impossible to say that any model contains no flaws and to say that a model accurately represents a natural system is theoretically impossible (Oreskes et al. 1994).

However, models can be confirmed when the predictions of the model match the observational data. While this does not imply truth or legitimacy, it does suggest that the model is approaching these goals. The probability that the model is not flawed is improved with increasing diversity and numbers of confirming observations. Yet, because more than one theory could explain the observations and since confirmation now does not guarantee confirmation in the future, any hypothesis tested using a model cannot be verified or validated, only confirmed (Oreskes et al. 1994).

This is not to say that models are not useful for they often enable the testing of ideas and development of new ones based upon results. This was true in the development of the community conditioning hypothesis through microcosm studies. A system like the SAM is a very simplified, generic, physical model of freshwater systems but it contains aspects of the complexity of the interactions found in natural freshwater systems (Cairns and Cherry 1993). The SAM is also an irreversible system (Landis et al. 1993a, 1994) - another property maintained by complex systems (Nicolis and Prigogine 1989). The simplicity of the SAM makes detection of changes in dynamics between treatments following disturbance easier (Taub 1984). As long as the limitations of models are not forgotten, systems like the SAM can provide great insight into the response of complex systems to disturbances.

Complex Data Sets

The complex, hypervariate data sets created by SAM studies present a challenge in data interpretation. While univariate methods can reveal patterns for individual variables, they do not address community-level responses. Moreover, there are no natural endpoints for this level of response (Landis et al. 1994). Attempts at addressing this problem include the development of indexes such as species diversity and the Index of Biotic Integrity (Karr 1993). However, when using an index one assumes to know the important variables to measure in the system at any given time. Also, indexes collapse all of the information present in many variables down into a single value. A great deal of information is lost in this process. Differences in variability and the distribution of organisms in the community become hidden as do the dynamics of the system. Indices also assume that there is a certain index value associated with an undisturbed system or a system in equilibrium (Landis et al. 1994).

Yet, more and more evidence is suggesting that naturally occurring ecological systems are not in equilibrium. This means that there is no baseline by which to judge ecological systems because they are dynamic and always in a state of change (Reice 1994). Consequently, characterizing a community with a single number or index and evaluating it in a linear manner from "healthy" to "unhealthy" is not appropriate (Suter II 1993; Landis et al. 1994).

Modern computerized tools can help to alleviate the problems associated with indices and other conventional data analysis methods when dealing with multivariate data sets. One such tool is nonmetric clustering and association analysis as developed by Matthews and Hearne (1991) designed specifically for the analysis of complex ecological data sets (Matthews et al. 1994, 1995). This artificial intelligence (AI) tool has been used successfully in several ecological (Matthews et al. 1991a, 1991b) and toxicological (Landis et al. 1993a, 1994) studies. Nonmetric clustering and association analysis was used in conjunction with conventional multivariate techniques in the present study.

Materials and Methods

Reagents

Chemicals used in the culture of test organisms, formulation of microcosm media, and reference toxicity tests were reagent grade or as specified by ASTM (ASTM E1366-91 1991). Hydrocarbon reference standards used in the identification and quantification of JP-8 components in microcosm samples were A.C.S. spectrophotometric grade ($\geq$99+% purity) and purchased from Alltech Chemical Company (Deerfield, IL). The ASTM D3710 Qualitative

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Calibration Mix (ASTM D3710-86 1986) and the ASTM D2887 Qualitative Reference Reformate Standard (ASTM D2887-86 1986) were purchased from Supelco Chromotography Products (Bellefonte, PA). A.C.S specification hexane or carbon disulfide purchased from VWR Scientific (Seattle, WA) was used in the preparation of all standards.

Glassware

All glassware was washed using Labtone® nonphosphate soap and hot tap water. This included glassware used in the culture of laboratory organisms; in the preparation of reagents, solutions, and microcosm media; and in the preparation of microcosm containers, samplers, and sample reservoirs. Glassware was rinsed in hot water until all soapy residue was removed, rinsed ten times in distilled water, and inverted and allowed to dry. Microcosm containers, samplers, and sample reservoirs were also acid soaked in 2N HCl for a minimum of fifteen minutes. Glassware was then rinsed ten times in distilled water and inverted to dry. Aluminum foil (dull side down) was used to cover all glassware prior to autoclaving for twenty minutes in a Market Forge Sterilemaster at 15 psi and 250° C. See Markiewicz (1994) for chromotography glassware procedures.

Class A volumetric glassware was used in all reagent preparation, measuring, and dispension for short term toxicity testing. Separatory funnels were used for preparation of the water soluble fraction of the jet fuel. Class A graduated cylinders were used in the measuring and dispensing of the water soluble fraction (WSF) of JP-8 during the dosing process in an effort to limit the volatilization of the jet fuel.

Water Soluble Fraction

The WSF of JP-8 was prepared in separatory funnels that were washed in Labtone® nonphosphate soap, rinsed in tap water, soaked in 2N HCl for at least one hour, rinsed ten times in distilled water, dried and autoclaved for twenty minutes in a Market Forge Sterilemaster at 15 psi and 250° C. JP-8 was supplied by the United States Air Force Toxicology Laboratory at Wright Patterson Air Force Base in Dayton, Ohio.

Using the microcosm media T82MV as the dilutent, WSF was made the day prior to dosing by adding 25 mL of JP-8 to one liter of fresh, sterile T82MV in a 1 L separatory funnel. This mixture was shaken vigorously for five minutes, releasing built up pressure as necessary. The contents were then allowed to stand undisturbed for 15 minutes. This pattern was repeated for a total time of one hour. At the end of this mixing period, the separatory funnel contents were allowed to remain undisturbed for eight hours, checked for air bubbles, and allowed to stand overnight. The next day all but 100 mL of the contents in the separatory funnel was drained into a clean, sterile Erlenmeyer flask (leaving the lighter insoluble fraction) and used immediately. A sample of the WSF was also drained into a clean, sterile one liter amber glass bottle and capped with a Teflon®-lined screw cap. This sample was archived at 2° C.

Microcosm Construction

Microcosms were constructed in accordance with the SAM protocol (ASTM E1366-91 1991) with modifications: (1) the length of the experiment was extended from 63 to 91 days, (2) Paramecium bursaria were substituted for Hypotrichs, and (3) a second stressor was added to the design. Table 1 outlines the SAM conditions.

As indicated by ASTM (ASTM E1366-91 1991) and described previously by Landis et al. (1993a, 1994), thirty microcosms were prepared in 1 gallon glass jars purchased from Richards Packaging (Kent, WA). Each jar contained 3 L of chemically defined sterile medium (T82MV) and sterile sediment consisting of 200 g silica sand (Fred Meyer, Bellingham, WA), 0.5 g ground chitin (J.T. Baker Co.), and 0.5 g cellulose powder (J.T. Baker Co.). The microcosms were arranged in a randomized block design on a light table with illumination of 80 mE m^{-2} photosynthetically active radiation s^{-1} and a 12:12 day-night cycle. The temperature of the room was maintained at 22 ± 2° C.

On day 0 of the 91-day experiment, nine algal species were inoculated at an initial concentration of 10^3 cells/mL for each species. On day 4, three macroinvertebrate, two microinvertebrate, and one bacterial species were inoculated at densities specified in the SAM protocol (ASTM E 1366-91 1991). On day 5, pH (ATC pH Wand) and dissolved oxygen (DO; YSI Model 57 Oxygen Meter) measurements were taken before dawn and in the evening. On day 6, before dawn pH and DO readings were again recorded. All pH and DO readings from days 5 and 6 were entered into a spreadsheet where the mean and standard deviation were calculated. These values as well as a visual inspection of all microcosms were used to determine culls and selection of the 24

Experimental Design

Each of the 24 microcosms used in the test was randomly assigned to one of four treatment groups (**Table 1**). In each experiment this resulted in six replicates for each treatment. Treatment 1 served as the reference system. Treatments 2 and 3 served to evaluate the effects of heat or jet fuel alone, respectively. Treatment 4 served to evaluate both stressors in sequence.

Table 1. Stressor Regime for the Microcosm Experiments

JP-8/HeaExperiment

TreatmentNo.	JetFuel	HeatShock
1	None	None
2	None	35° C
3	15% WSF	None
4	15% WSF	35° C

Jp-8/JP-8Experiment

TreatmentNo.	JetFuel	Jet Fuel Again
1	None	None
2	15% WSF	None
3	15% WSF	15% WSF
4	None	15% WSF

There are several notable aspects of this design. The jet fuel JP-8 is known to cause significant changes in SAM community dynamics at 15% WSF. Also, the heat stressor occurred on day 33 when a convergence is often seen between the reference group and the jet fuel treated groups (Landis et al. 1993a, 1994). Markiewicz (1994) has shown that the jet fuel is undetectable in the water column by this time in SAMs, indicating that the jet fuel is rapidly degraded. Finally, the length of the JP-8/heat shock SAM was extended from 63 to 91 days because previous extended SAM studies have demonstrated that important changes in community dynamics do occur after day 63. In the JP-8/JP-8 experiments the first second dosing was on day 63 and the experiment continued until day 120.

Microcosm Sampling and Dosing

Prior to dosing with WSF on day 7 and for the remainder of the test period, turbidity (Hach Model 2100), DO, pH, and number of each inoculated species (excluding bacteria) were determined twice weekly. Room temperature, table illumination, and the day/night cycle were maintained at constant values throughout the test period. Reinoculation of algae, macroinvertebrates, and microinvertebrates occurred once per week per the SAM protocol (ASTM E1366-91 1991).

After all biological counts were complete on day 7, 450 mL of media were removed from each microcosm using sterile Nitex® 100 mesh to prevent removal of the organisms in the

microcosms. In the reference and heat only treatments, this removed volume was replaced with 450 mL of fresh T82MV. In the JP-8 and JP-8/heat treatments, the volume was replaced with 450 mL of WSF of JP-8. Each microcosm was then stirred.

On day 33, the microcosms in treatment groups 2 and 4 were placed in an environmental chamber (pH Environmental Model CEC 50LTP) at 35° C for 8 hours. Following the heat stress period, the microcosms were returned to the light table and the temperature of each microcosm was recorded.

Once per week beginning on day 11, two 50 mL samples were taken from each microcosm for nitrate and soluble reactive phosphorus (SRP) analysis. Samples were removed using sterile 60 mL plastic syringes (Becton-Dickinson) and filtered though an acid washed Gelman 0.45 mm Metricel® membrane filter. All samples were stored in acid washed Nalgene plastic bottles and stored at 3° C until analyzed.

From day 7 through day 10, 9.5 mL were removed from each microcosm every 12 hours and placed in sterile test tubes with Teflon® lined caps such that there was no airspace. This volume was replaced with 9.5 mL of fresh T82MV. Samples for alternating groups of 12 microcosms (#1-12 or #13-24) were kept for analysis while the remaining 12 samples were discarded. Beginning on day 11 and continuing through day 28, samples were taken from all microcosms in the evening only. Again, alternating groups of 12 samples were kept for analysis and the remaining 12 discarded. On day 35, additional samples were taken from all microcosms for post heat stress analysis. All samples intended for analysis were stored at 2° C until analyzed.

Several changes were made in the constructiona and sampling of the JP-8/JP-8 experiment. These changes are summarized in **Table 2**.

Data Analysis Methods

During the test period, all data were recorded onto computer entry forms, entered into a spread sheet program, and checked for accuracy. Data were converted into an ASCII file so as to be compatible with SPSS-X data file format and with the AI applications.

Univariate Methods

Scatterplots and the frequency distribution of each variable over time by treatment were plotted using SPSS-X. Because most parameters did not meet the normality requirement of Analysis of Variance (ANOVA), all data were transformed ($\log_{10} (X+1)$). This univariate statistical approach was used as specified in the SAM protocol (ASTM E 1366-91 1991) to determine the significance of the collected parameters in treated groups as compared to the reference treatment. Tukey's Honestly Significant Difference multiple range test (a=0.05) was then conducted for days which exhibited significant differences in the ANOVA analysis (a=0.05). ANOVA and multiple comparison tests were performed using SPSS-X.

Multivariate Methods

In addition to univariate approaches, hierarchial clustering was performed with SPSS-X using Euclidean and cosine methods with single and complete linkages on raw data. This agglomerative process progressively builds clusters or groups of similar sampling units (SUs) until all SUs are joined into one group. In the case of the SAM, these sampling units are the microcosms. The resulting dendrogram is used to evaluate the presence or absence of significant clustering by the clusters' relative distance from one another. It is up to the analyst to determine what constitutes a significant clustering (Ludwig and Reynolds 1988). The hierarchial clustering program does not include a significance test to evaluate whether clusters and treatments coincide, thus, the interpretation of the results is left to the analyst.

In addition to hierarchial clustering, two metric multivariate significance tests were employed that have been extensively used in other SAM experiments (Landis et al. 1993a, 1994). One test computed the multivariate metric distance using Euclidean distance and the other used cosine of vectors distance (Good 1982; Smith et al. 1990). The distances within and between treatments were computed using raw data. Using an approximate randomization test (Noreen 1989), the

Table 2. Comparison of the double stressor microcosm experiments.

	JP-8/Heat Shock	JP-8/JP-8
Organisms/Microcosm Algae (added on day 0 at initial concentration of 10^3 cells/mL for each algal species):	*Anabaena cylindrica, Ankistrodesmus falcatus, Chlamydomonas reinhardi 90, Chlorella vulgaris, Lyngbya sp., Scenedesmus obliquus, Selenastrum capricornutum, Stigeoclonium sp., Ulothrix sp.*	
Animals (added on day 4 at the initial numbers indicated in parentheses):	*Daphnia magna* (16 per microcosm) *Cypridopsis sp.* [ostracod] (6 per microcosm) *Paramecium bursaria* [protozoa] (90 per microcosm) *Philodina sp.* [rotifer] (90 per microcosm)	
Experimental Design Test vessel type and size	One gallon (3.8 L) glass jars 16.0 cm wide at the shoulder, 25 cm tall with 10.6 cm openings	NC
Medium volume:	3 L added to each test vessel	NC
Number of replicates x concentration:	6x4	NC
Reinoculation	Once per week add two drops (ca. 0.1 mL) to each microcosm from a mix of the nine species of algae and microinvertebrate culture tubes. Also add macroinvertebrates once per week as needed to bring sample count to 3 for each species.	
Stressor regime:	WSF of JP-8 added on day 7 by removing 450 mL from test container and adding 450 mL of WSF to produce test concentration of 15% WSF. Heat shock for 8 hours at 35° C on day 33.	WSF of JP-8 added on day 7 by removing 450 mL from test container and adding 450 mL of WSF to produce test concentration of 15% WSF. Additional JP-8 stressor added on day 33 in the same fashion as first treatment.
Sampling Frequency	2 times a week	
Test Duration	91 days	
Physical and Chemical Parameters		
Temperature	20-25 ° C	NC
Light Intensity	80 mE m^{-2} photosynthetically active radiation s^{-1}	NC

Photoperiod	12 h light/12 h dark	NC
Medium	Medium T82	NC
Sediment	Composed of silica sand (200g), ground, crude chitin (0.5g), and cellulose powder (0.5g) added to each test container	NC
Measurements	Algal, microinvertebrate, and macroinvertebrate counts, pH, dissolved oxygen, turbidity, nitrate, soluble reactive phosphorus. Parameters calculated included concentrations of each species.	NC

ratio of within treatment distances to between treatment distances was used to evaluate whether an association with treatment was present. A more detailed summary of this process as it applies to microcosms may be found in Landis et al. (1994).

Additionally, a nonmetric clustering association test was employed to examine community dynamics. Nonmetric clustering and association analysis (NCAA) was developed by Matthews and Hearne (1991) specifically for evaluating complex ecological data sets (Matthews et al. 1994, 1995). NCAA, an artificial intelligence (AI) technique, is a clustering program that finds the best clustering for a data set using a conceptual model. Variables on a given sampling day are evaluated categorically (nonmetrically) enabling the use of data with different distributions, unequal variances, presence/absence data, and a variety of measurement types. NCAA does not require transformations of the data. The clustering is conducted naive to treatment group and, once finished, an association analysis is performed to see if clusters correspond to treatment groups. This process utilizes Pearson's c^2 test. When a significant association occurs this indicates that with the given data we can predict which treatment a microcosm belongs to with accuracy greater than chance. This approach has also been described in its application to the SAM by Landis et al. (1993a, 1994) and Matthews et al. (1994, 1995).

The NCAA program Riffle begins an analysis at a point designated by the 6-digit random seed that is chosen by the analyst. Riffle then finds the best clustering based upon the variables that best describe differences between the clusters. The actual number of variables used by Riffle in the analysis is also chosen by the analyst. Riffle repeats the analysis the number of times indicated by the number of retries and the output shown is the best clustering found in the analyses. For the current research, analyses for both two and four clusters were run with ten to twenty significant features as selected by the NCAA program, 10 retries per analysis, and up to 3 random seeds.

Visualization Tools

In addition to the above techniques, the data were inspected using a visualization tool developed by Matthews (1992) known as space-time worms. The Worm.app program as used in the analysis process was developed by Roze (1992). This visualization process involves projecting two variables over time in a three dimensional graphical display. For any given sampling day, the mean for each treatment group is plotted. Around this mean is a circle with a radius proportional to the average distance between replicates within the group. The plots for each sampling day are then connected to give a worm-like projection showing the dynamics of the two plotted variables over time for each treatment. The projection may be rotated in three dimensional space to obtain different perspectives of the image. Space-time worms enable comparison of the dynamics of treatment groups over time and give an indication of the trajectories relative to each other.

Microcosm Results

JP-8/Heat Results
Univariate Results

The most notable pattern obtained with univariate analysis of the data was the striking similarity among the reference and heat only treatments and among the JP-8 and JP-8/heat treatments throughout the length of the experiment. This was particularly clear in scatterplots of the data and also a common theme in the ANOVA results, although some variables such as *P. bursaria* and small amphipods exhibited more varied dynamics. All figures below are constructed with raw data and error bars are standard error of the means for each sampling day.

Algal Dynamics

JP-8 treated microcosms exhibited the usual algal bloom after the near extinction of daphnid populations. Thus, JP-8 and JP-8/heat treatments had significantly more algae than the reference

and heat only treatments. However, differences between treatments were undetectable for most species of unicellular green algae after day 28, just prior to the heat stress on day 33.

In contrast to this pattern, *Scenedesmus obliquus* continued to display treatment related differences for the length of the experiment. Through day 42, multiple comparison tests show that the quantity of algae in the JP-8 dosed treatments was statistically greater than that in the reference and heat only treatments. From days 42 to 74 the amount of algae in the JP-8/heat treatment was consistently significantly greater than that in the reference and heat only treatments. After day 74, both the JP-8 and JP-8/heat treatments again had significantly greater amounts of *S. obliquus* compared to the reference and heat only treatment. **Figure 4** shows these results graphically and also shows that the means of the JP-8 and JP-8/heat treatments are consistently distinct after day 25, although these differences are not statistically significant with ANOVA.
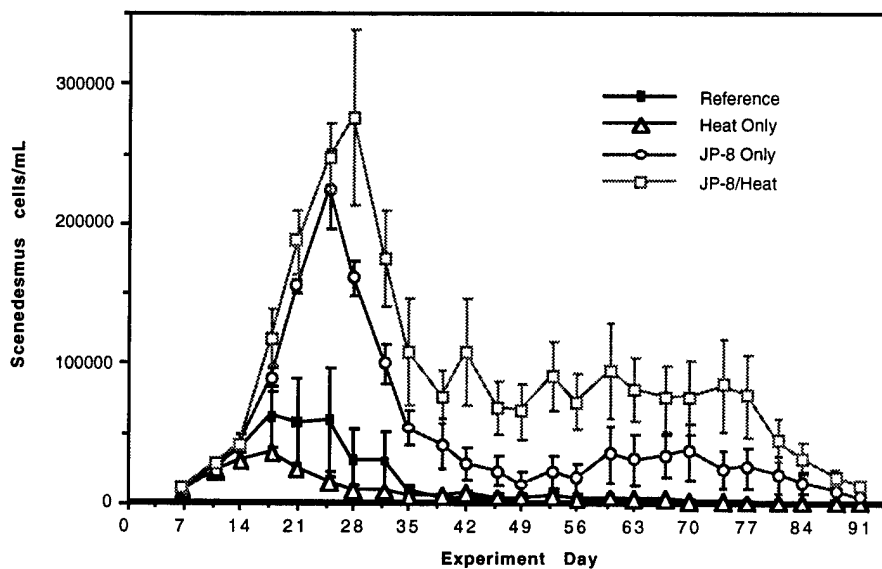
Figure 4. *S. obliquus* population dynamics. Quantities of *S. obliquus* in JP-8 treated microcosms are consistently greater than in the reference and heat only treatments.

A. falcatus, another unicellular green algae, exhibited a second pattern of significant differences from day 39 to 60 when the quantity of algae in the JP-8 and JP-8/heat treatments was often greater than that of the reference and heat only treatments according to ANOVA and Tukey's test. However, the treatments that displayed differences varied by sampling day in multiple comparison test results. The jet fuel/no jet fuel distinction returned from day 81 to 91 but again varied in specific significant differences by sampling day. **Figure 5** shows these results graphically and suggests that from days 81 to 91 the reference and heat only treatments were distinct from the JP-8 and JP-8/heat treatments.

For the blue-green algae *Anabaena cylindrica*, JP-8 dosed groups often had statistically greater quantities of the algae than the nondosed groups with particular differences varying by sampling day. This trend began around day 18 and continued through day 63. The distinction is particularly evident after day 32. These results are presented in **Figure 6**.
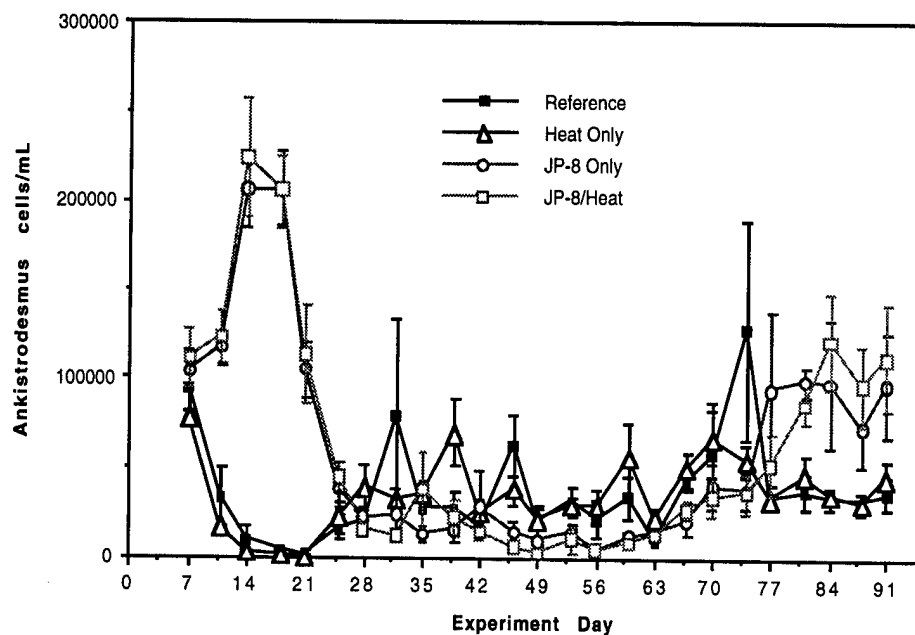
Figure 5. *A. falcatus* population dynamics. JP-8 treated groups show greater quantities of *A. falcatus* than the reference and heat only treatments from day 11 to 32 and day 81 to 91.
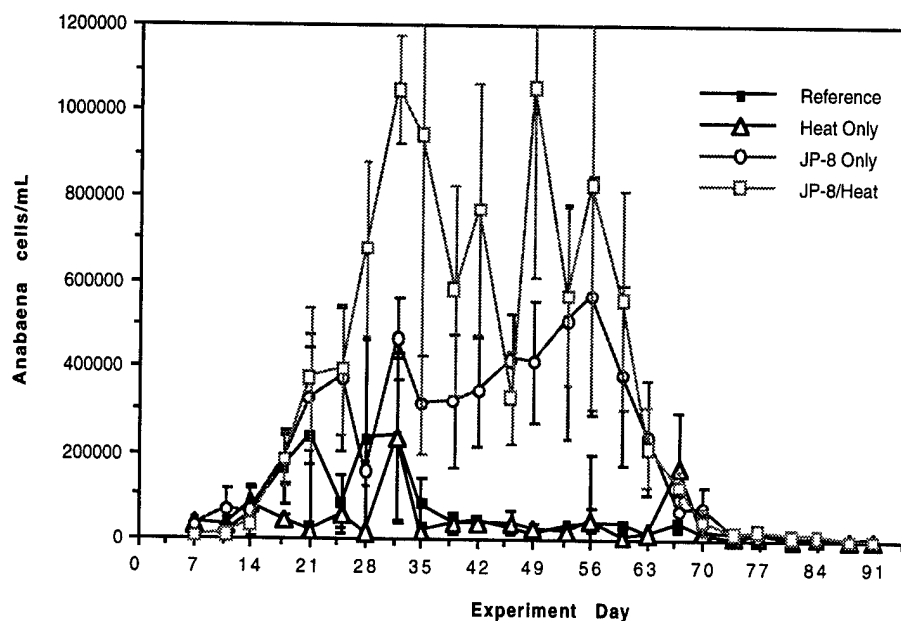
Figure 6. *A. cylindrica* population dynamics. After day 32 and through day 63, the distinction between the jet fuel dosed and nondosed treatments is most apparent.

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Macroinvertebrate Dynamics

Daphnid populations were drastically reduced in the JP-8 treated groups as compared to nondosed groups (**Figures** 7 and 8). These differences were statistically significant until just before the heat stress. After the heat stress, the populations of medium *D. magna* in the reference and heat only treatments again displayed increased numbers compared to those in the JP-8 and JP-8/heat treatments but were only statistically significant on days 46, 49, and 56 (**Figure** 7). Small *D. magna* populations show statistically significant differences between the reference treatment and both JP-8 dosed treatments from day 81 to 91. Relatedly, during the period from day 53 to day 74, ephippia production in the reference group was often statistically higher than in the JP-8 and JP-8/heat treatments.

Amphipods began to exhibit treatment related differences by day 18 when the heat only treatment showed statistically significant increased numbers in small amphipods in comparison to JP-8 and JP-8/heat treatments. This trend continued until the heat stress on day 33.

After the heat shock, the heat only treatment demonstrated statistically higher counts for medium amphipods than the jet fuel treated groups though day 81. Beginning on day 70, the reference group also had significantly fewer medium amphipods than the heat only treatment. Similarly, after the heat stress, the heat only treatment had statistically higher counts for small amphipods than all other treatments. This trend continued through day 91. Also, after day 60, the reference treatment often had statistically greater numbers of small amphipods compared to the jet fuel treated groups. **Figure** 9 shows small amphipod populations by treatment. With the exception of day 84, the means of the small amphipod populations in all four treatments appear graphically distinct after day 74.

Ostracod populations increased in all treatments over the length of the experiment. However, few significant differences were detected between treatments. When distinctions were present, there was no obvious trend in the differences. It is notable that the jet fuel/no jet fuel distinction seemed to be present at the end of the experiment, with slightly elevated numbers of ostracods in JP-8 and JP-8/heat treatments.
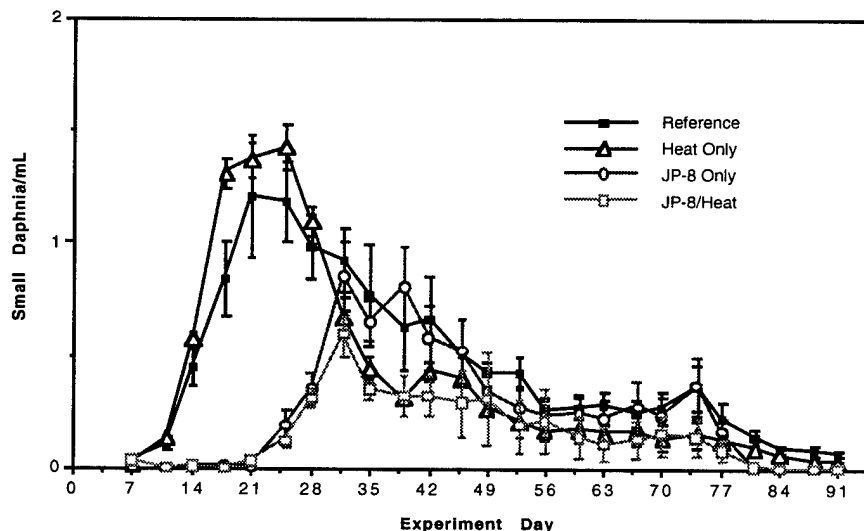
Figure 7. Small daphnid population dynamics. Reference and heat only treatments display similar dynamics from day 11 to 32 as do JP-8 and JP-8/heat treatments. After day 32 all treatments follow a similar pattern.

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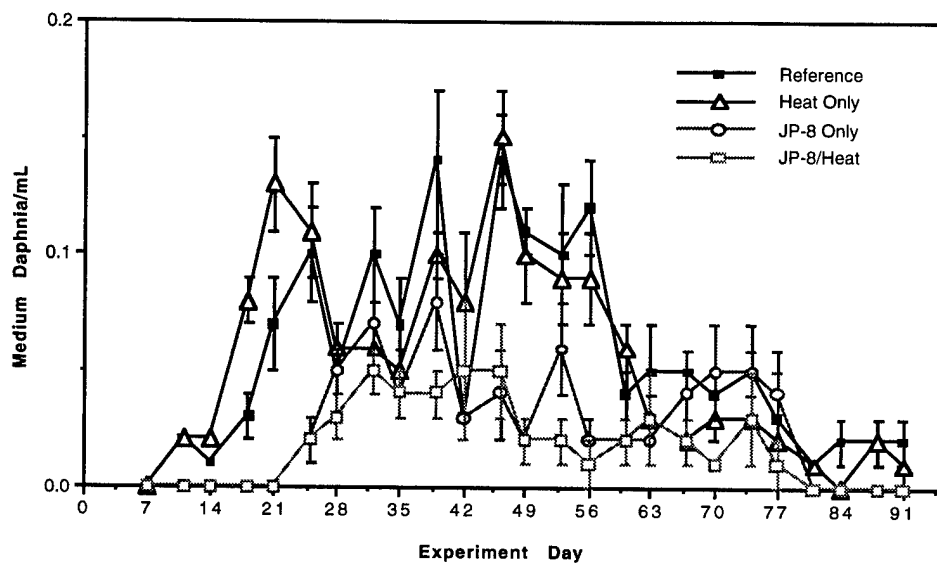
Figure 8. Medium daphnid population dynamics. Reference and heat only treatments display similar dynamics over the course of the experiment as do JP-8 and JP-8/heat treatments.
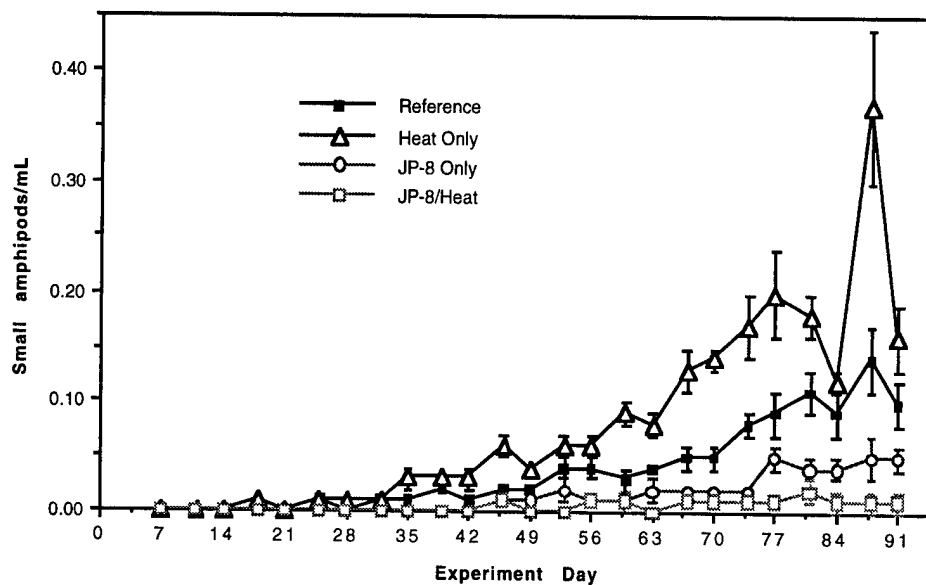
Figure 9. Small amphipod population dynamics. Treatments appear distinct after day 63, but differences are only statistically significant for the heat only treatment and the reference treatment.

MicroinvertebrateDynamics

Microinvertebrate results differed for each species. *Philodina* populations showed very little treatment related response. Populations in all treatments followed the same general trend of increase through day 56 followed by a decrease through the end of the experiment (**Figure** 10). When differences were detectable, multiple comparison tests show varied response although differences were always between a JP-8 dosed and nondosed group. Results presented in **Figure** 10 do suggest that the reference and heat only treatments contained greater quantities of *Philodina* than the JP-8 and JP-8/heat treatments from days 56 through 67.

As for *P. bursaria*, a distinct pattern from day 18 to 28 suggests differences between JP-8 dosed and nondosed groups with elevated numbers of *P. bursaria* in the treated microcosms. After a long period of similarity, days 60 to 74 show the JP-8/heat treatment to have statistically greater numbers of *P. bursaria* than the reference and heat only treatments. A similar pattern is evident from days 84 to 91 when the JP-8/heat treatment has statistically greater numbers of *P. bursaria* than the reference treatment. Graphically, the means of all four treatments appear very distinct at the end of the test (**Figure** 11).

Trends in pH

Significant differences in pH were detectable on all sampling days but days 60 and 63. For the days prior to the heat shock (days 7 to 28), the differences were between treatments that were not dosed with jet fuel and those that were, with pH in the treated microcosms much higher than in the reference and heat only treatments. From day 35 to day 56 differences were more varied with the heat only treatment often statistically higher in pH than the JP-8/heat treatment. By day 67 the no jet fuel/jet fuel treatment distinction returned and persisted for the remainder of the experiment but the pattern was not consistent until day 77. During this last period of differences, the reference and heat only treatments had statistically higher pH readings than the JP-8 dosed treatments (**Figure** 12).
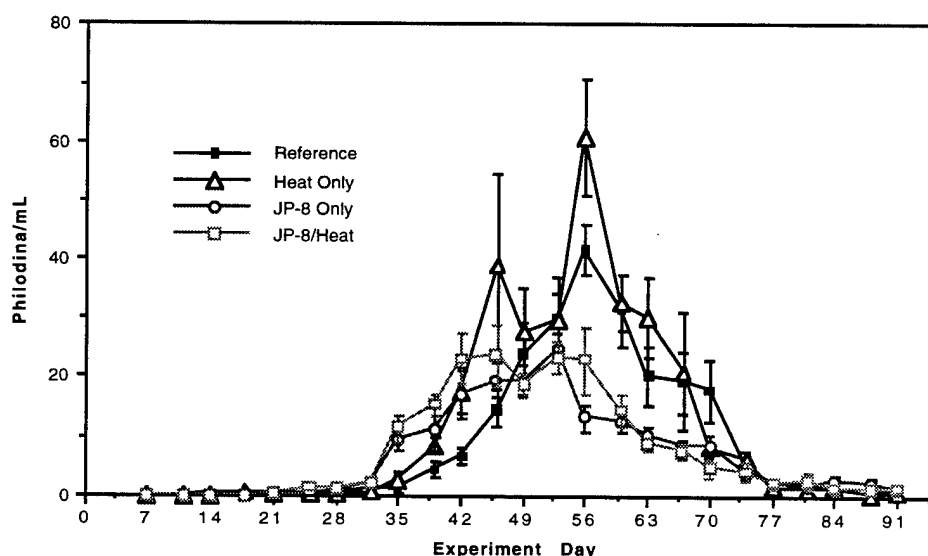
Figure 10. *Philodina* population dynamics. A treatment related response is suggested from day 56 to 67 where the reference and heat only treatments appear distinct from the JP-8 and JP-8/heat treatments.
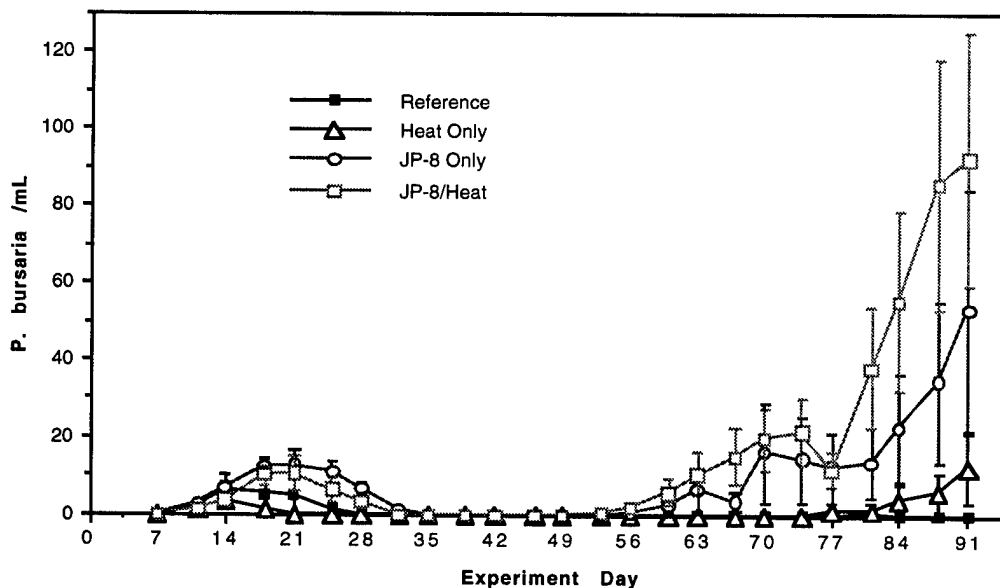
Figure 11. *P. bursaria* population dynamics. A JP-8 treatment effect is suggested in the first three weeks of the experiment. However, by the end of the test period, four treatments are suggested.
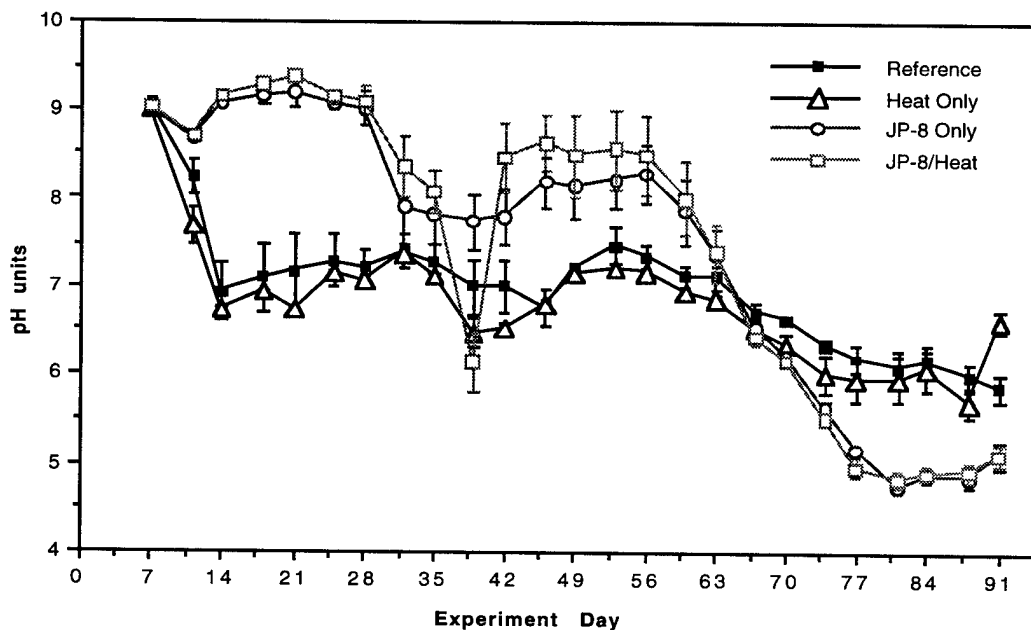
Figure 12. pH. The reference and heat only treatments displayed statistically significant differences from the JP-8 and JP-8/heat treatments for most of the test period.

Multivariate results

Hierarchial clustering of the data for each sampling day did not produce meaningful results. While a few moderate clusterings of two groups were seen early in the test period, they appeared to be distinguishing between sides of the microcosm table, not treatment groups. This clustering was not present on consecutive days nor did there seem to be a pattern to indicate that microcosm location exerted a strong influence on results.

MetricMultivariate Results

Euclidean and cosine vector distance analyses were conducted using the water quality and biological variables. The analyses searched for four groups on a given sampling day and ran with 1000 retries per analysis. Cosine results showed a strong pattern of significance from day 11 through 63 (**Figure** 13). Yet, even after day 63 significance results for the cosine analysis were often above 0.95. Euclidean results, however, did not show this lengthy pattern of significance. Rather, there were several periods with a significance greater than 0.95 separated by large drops in the significance. The significant periods were seen from days 7 to 18, 28 to 32, 46 to 53, and 70 to 81 (**Figure** 13).

Figure 14 shows the average Euclidean distance within the reference treatment and between the reference treatment and other three treatments plotted on a log scale. Euclidean distances showed little difference between groups prior to day 49 although a jet fuel/no jet fuel distinction is suggested. Treatments became more distinct through day 56. On day 81, the jet fuel/no jet fuel distinction returned. From day 84 to day 91, three groups were apparent: reference and heat only, JP-8, and JP-8/heat.
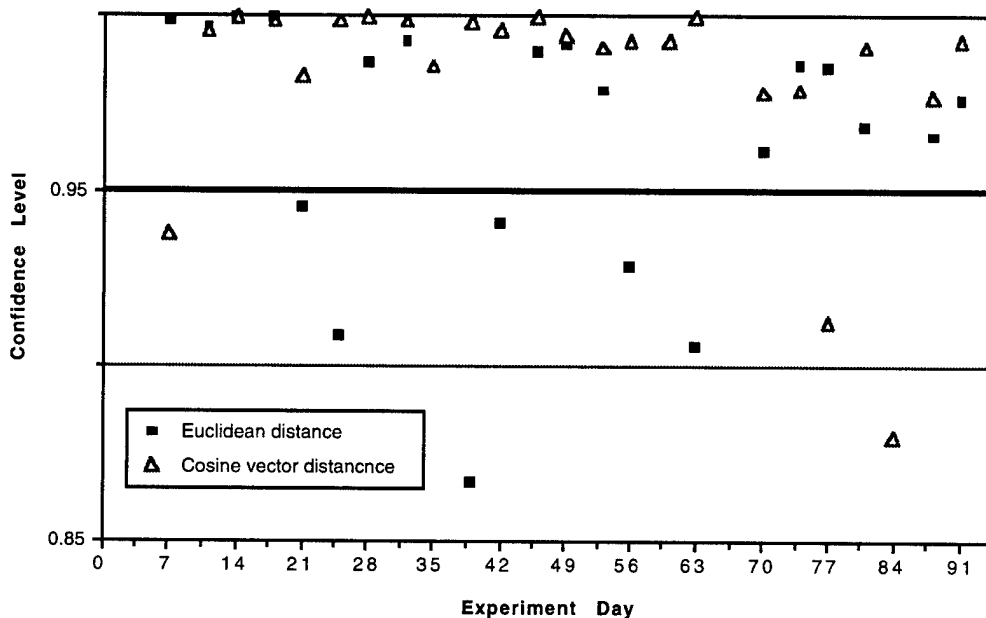
Figure 13. Significance levels for multivariate metric statistical tests for each sampling day. Significance for four treatments was high for most of the experiment with the cosine analysis. The 95% confidence level is indicated by the bold line.

The average cosine distances are plotted in the same manner in **Figure** 15. Contrary to the Euclidean results, two groups were very apparent just after dosing with JP-8. This distinction was between systems dosed with jet fuel and those that were not. There was more separation
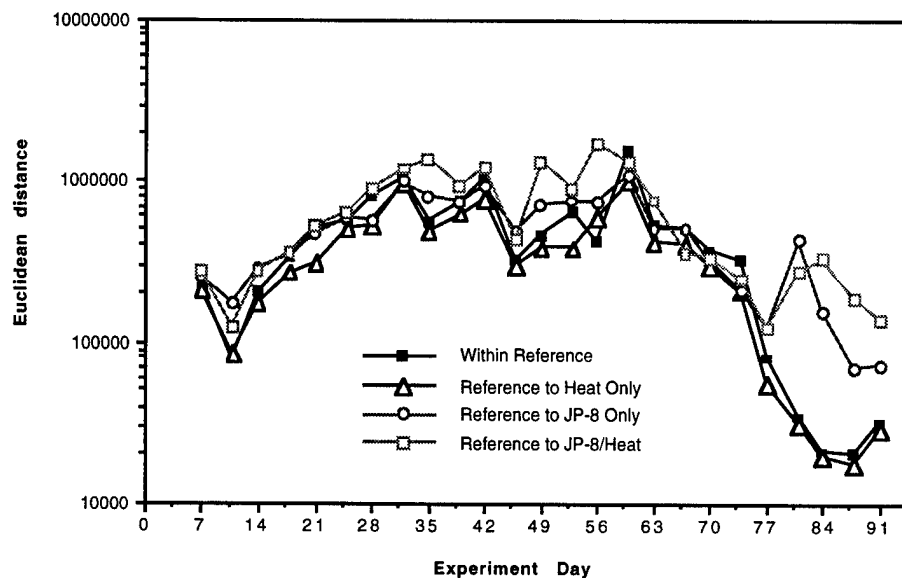
Figure 14. Euclidean distance within the reference group and from the reference group to each of the treatments for each sampling day. The largest differences were apparent after day 77 where three groups appear distinct.
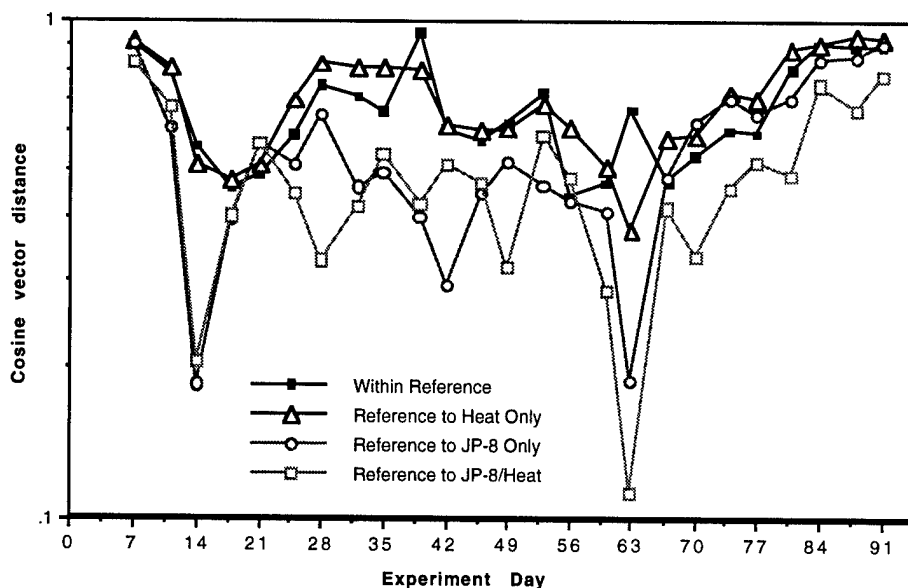
Figure 15. Cosine distance within the reference group and from the reference group to each of the treatments for each sampling day. Large differences wee apparent after dosing with JP-8. JP-8 and JP-8/heat treatments maintained large distances from the reference treatment for much of the experiment. After day 57, the JP-8/heat treatment was most distinctive.

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between all treatments after day 21. Often three and four treatments were distinct through the end of the test period. Moreover, the JP-8/heat treatment appeared very different from the rest of the treatments after day 67.

Nonmetric Clustering Results- Two Clusters

Nonmetric clustering and association analysis looking for two clusters for each sampling day was conducted using water quality and biological parameters. The analysis included 10 significant attributes as chosen by the Riffle program and 10 retries per analysis.

Cluster quality for two clusters was good throughout the experiment with few values below 0.6. Quality was highest just after addition of JP-8 to treatments 3 and 4. After peaking on days 18 and 21, the cluster quality showed periods of decrease and subsequent increase over the test period. Cluster quality remained fairly high at the end of the experiment. **Figure** 16 shows the cluster quality over time for this two cluster analysis.

Additional analyses for two clusters were conducted for the data on each sampling day using 20 significant attributes, 10 retries, and 3 random seeds to see if any improvement in results could be obtained by using additional variables in the analysis. Results showed fair clustering on most sampling days after addition of JP-8; however, the quality was always less for 20 attributes than for 10 (**Figure** 17). Cluster quality showed the same pattern as the first analysis, with an increase in cluster quality after dosing with JP-8 that is followed by a sequence of decreases and increases in cluster quality with highest cluster qualities occurring around days 56 and 88.

The association analysis results demonstrated that the two clusters were strongly associated with jet fuel treatment throughout most of the experiment in both the analysis with 10 significant attributes and the analysis with 20 significant attributes (**Figure** 18). Reference and heat only treatments were grouped together in the contingency tables as were the JP-8 and JP-8/heat treatments. In fact, there was almost no difference between the association analyses using 10 significant attributes and 20 significant attributes. Consequently, because the cluster quality was better for the first analysis using 10 significant attributes, the results from that analysis will be used in subsequent discussions of two cluster results.

When decreases in significance were present for the two cluster analysis, they were associated with decreases in cluster quality (**Figure** 19). The confidence level dropped below 95% on a few sampling dates and always returned to near 1.0 after each of these decreases. This was particularly notable at the end of the 91-day test period when the confidence level was 0.99.

Nonmetric Clustering Results - Four Clusters

The Riffle program was also used to look for four clusters in the data. Results from this analysis using water quality and biological parameters (10 significant attributes and 10 retries) showed good clustering throughout the entire experiment after dosing with JP-8. Cluster quality was above 0.6 for most of the test period. Days 63 to 77 showed a decrease in cluster quality, but never was it below 0.51. After this period, the quality improved and remained high through the end of the experiment (**Figure** 20). As with two clusters, the association analysis showed a high significance value, suggesting an association between clusters and treatment group through most of the experiment (**Figure** 20).
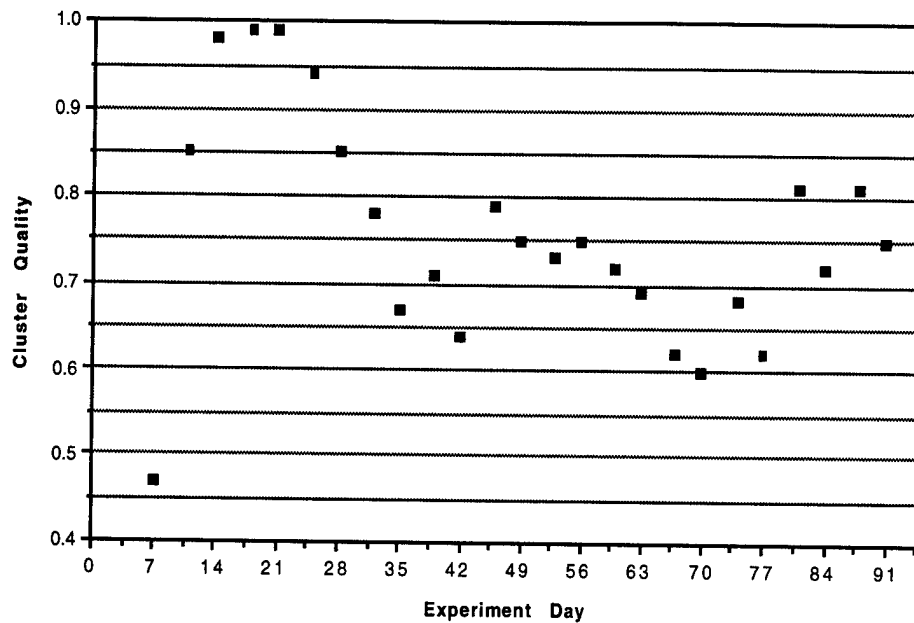
Figure 16. Cluster quality for two clusters on each sampling day with NCAA using 10 significant attributes. Quality was very high after dosing with JP-8 and remained good for the length of the experiment.
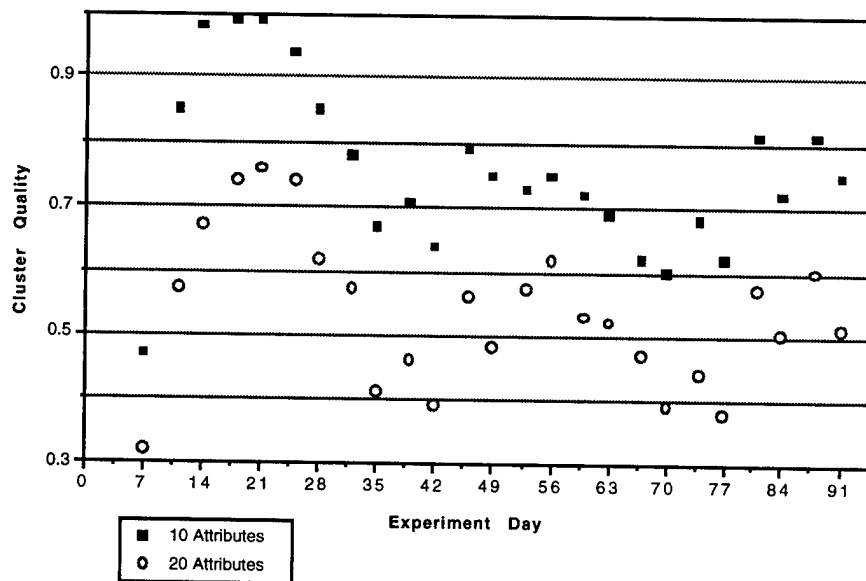
Figure 17. Cluster quality for two clusters on each sampling day with NCAA using either 10 or 20 significant attributes in the analysis. Cluster quality was similar for either analysis but always higher for the analysis with 10 attributes.
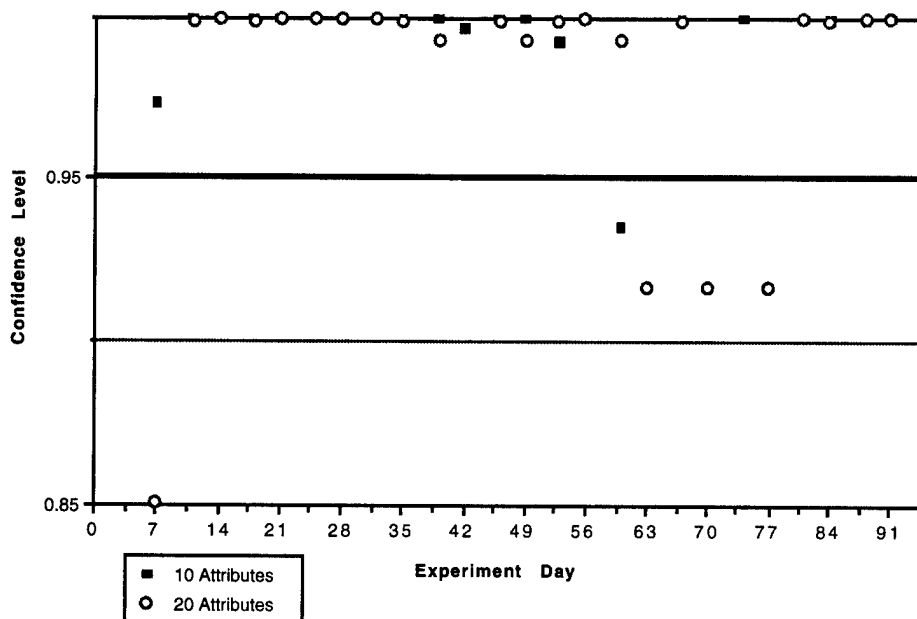
Figure 18. Significance levels for two clusters on each sampling day with NCAA using either 10 or 20 significant attributes in the analysis. Results were similar for both analyses, showing clustering into treatment groups at or above the 95% confidence level on most days.
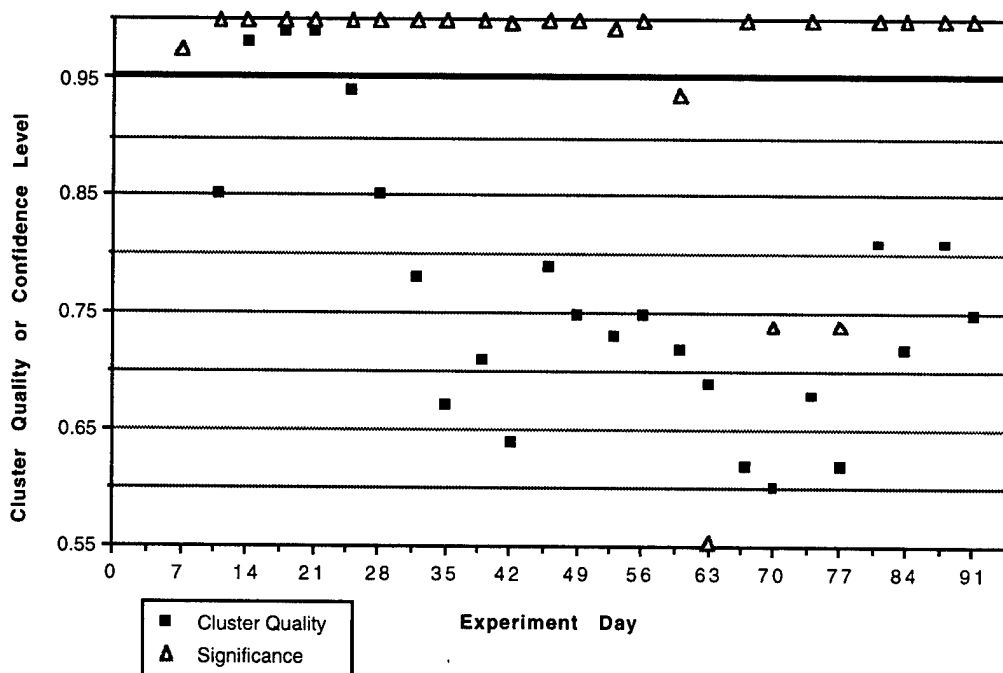
Figure 19. Cluster quality and significance levels for two clusters on each sampling day with NCAA.
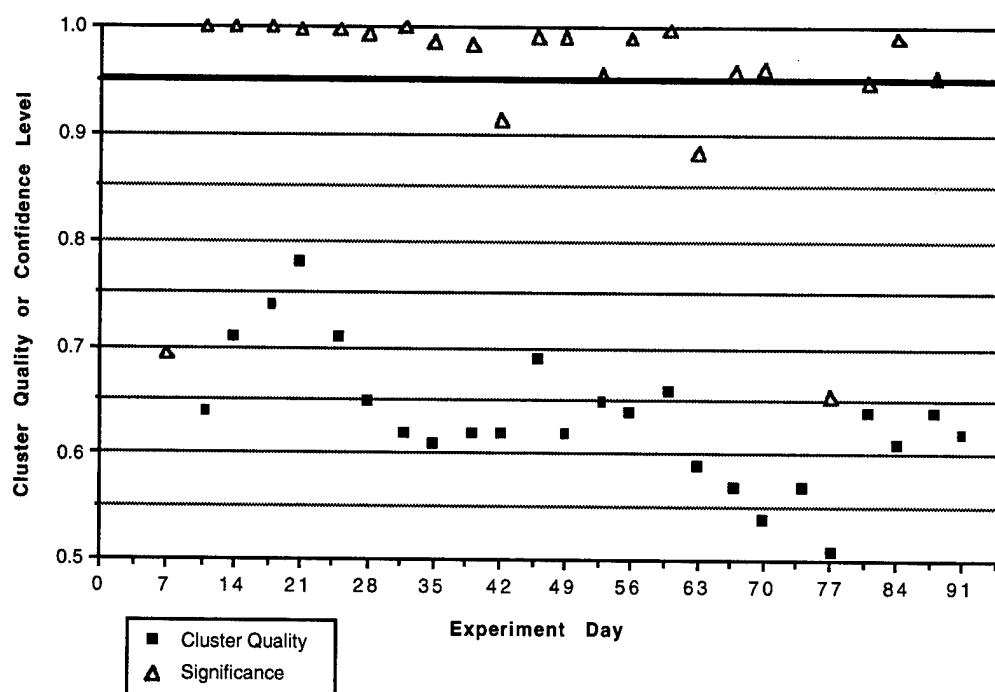
Figure 20. Cluster quality and significance levels for four clusters on each sampling day with NCAA. Clustering into treatment group was significant at the 95% confidence level or above for most of the experiment.

On any given sampling day, cluster quality was always higher for two clusters (**Figure** 21) but the pattern of increase and decrease in cluster quality was the same for either two or four clusters. Results from the association analysis were very similar for either two or four clusters (**Figure** 22) but the significance values were usually higher for 2 clusters.

Significance results from the metric analyses were very similar to those from NCAA, with cosine vector distances showing the greatest similarity to NCAA. These results are shown in **Figure** 23.

Important Variables in the NCAA Analysis

For days 11 through 32, NCAA identified the water quality parameters, unicellular green algae, and D. magna as important in determining two and four clusters. After the heat shock on day 33, more of the biological variables became important in determining two clusters, specifically, A. cylindrica, and small and medium amphipods. This trend continued until day 67 when the biological variables became and remained the primary factors in two-cluster determination (Table 3).

For four clusters, the important variables after the heat stress also became more biological in nature although many water quality parameters remained important. Added to the list of biological variables are again amphipods and A. cylindrica. Beginning on day 53, P. bursaria was present in the list through the end of the experiment. After day 67, water quality parameters remained on the list of important variables but were usually ranked less than the biological variables (Table 4).
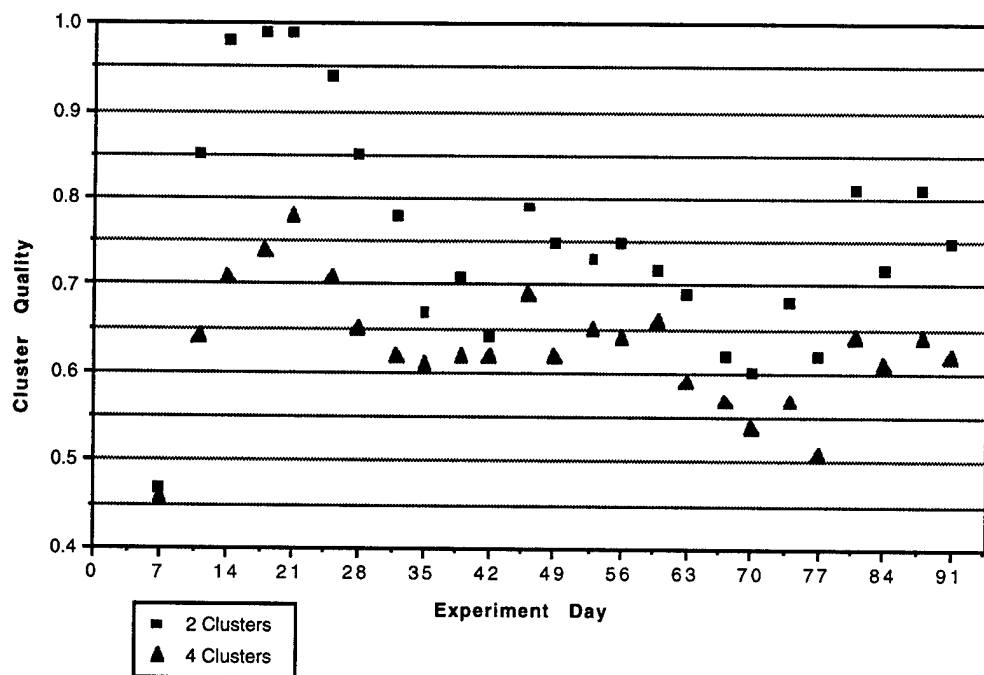
Figure 21. Cluster quality for two or four clusters on each sampling day with NCAA. Cluster quality was always higher for two clusters.
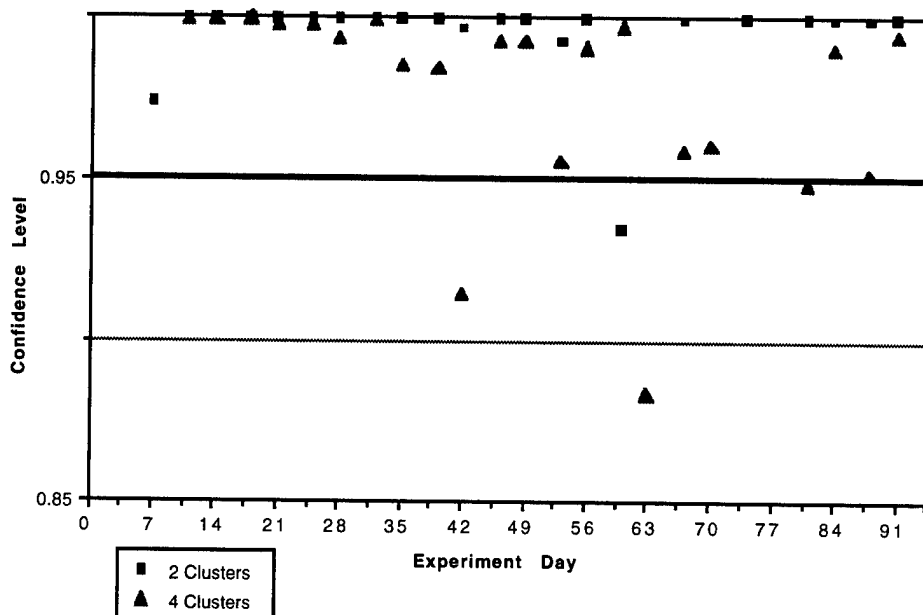
Figure 22. Significance levels for two or four clusters on each sampling day with NCAA. Both two and four clusters demonstrated confidence levels at or above 95% for much of the experiment.
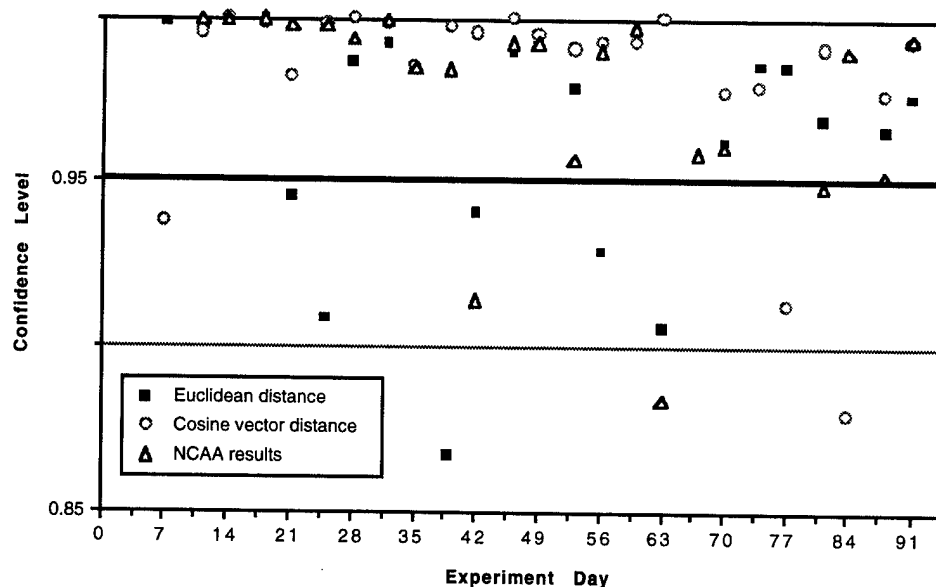
Figure 23. Comparison of significance levels for four clusters on each sampling day using the three multivariate statistical tests. NCAA and cosine results were very similar with clustering into treatment groups above the 95% confidence level for much of the experiment.

Table 3: Important variables for two clusters in the NCAA analysis[1]

Day	Variables in rank order
11	DO=large *D. magna*=medium *D. magna*=small *D. magna*[2,3] > *A. falcatus* > *S. capricornutum* > pH > medium amphipods
14	DO=pH=turbidity=large *D. magna*=medium *D. magna*=small *D. magna*= *A. falcatus* > *S. capricornutum*
18	DO=pH=medium *D. magna*=small *D. magna*=*A. falcatus*=*S. capricornutum*=*P. bursaria* > turbidity
21	DO=pH=medium *D. magna*=small *D. magna*=*A. falcatus*=*S. capricornutum*=*P. bursaria* > *S. obliquus*
25	DO=pH=phosphate=small *D. magna*=*S. obliquus* > *S. capricornutum* > large *D. magna*=small amphipods=turbidity
28	DO=small *D. magna* > pH=*S. obliquus*=*S. capricornutum* > small amphipods > *A. cylindrica*=turbidity
32	Turbidity > phosphate=*S. obliquus*=small amphipods > *A. falcatus*= ostracods > *A. cylindrica* > DO > nitrate
35	Ostracods > *S. obliquus* > small amphipods=turbidity > *A. cylindrica* > pH > *Philodina* > medium amphipods
39	*S. obliquus* > small amphipods=turbidity > ephippium > *A. cylindrica* > medium *D. magna* > *A. falcatus*=medium amphipods=*Philodina*=phosphate
42	DO > *C. vulgaris* > *A. cylindrica* > small *D. magna* > pH=small amphipods > turbidity > medium amphipods=*Philodina*
46	pH > *A. falcatus*=medium amphipods > *A. cylindrica*=small amphipods > *C. vulgaris*=*S. obliquus* > DO=medium *D. magna*

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49	*A. cylindrica*=small amphipods > medium amphipods > *C. vulgaris*=medium *D. magna* > *S. obliquus* > DO=*A. falcatus* > pH
53	DO=pH=small amphipods > ephippium > turbidity > *A. cylindrica* > *P. bursaria* > mediumamphipods
56	*C. vulgaris* > *A. cylindrica*=large amphipods=medium amphipods=medium *D. magna*=*Philodina*=DO=small amphipods=*Stigeoclonium* > *S. obliquus*
60	DO > pH > medium *D. magna* > *A. cylindrica* > ephippium > phosphate > *A. falcatus* > small *D. magna*
63	DO=ephippium=medium*D. magna* > small *D. magna*=*Stigeoclonium* > *A. cylindrica*=*P. bursaria*=pH > *Ulothrix*
67	*C. vulgaris* > small amphipods > *S. obliquus*=*C. reinhardi* > *A. falcatus* > phosphate=*S. capricornutum*
70	Small *D. magna* > medium *D. magna* > ephippium=*P. bursaria* > DO > *C. reinhardi*
74	Small amphipods > *C. vulgaris* > pH > medium amphipods > S. obliquus > nitrate > *A. falcatus*
77	Small *D. magna* > ephippium > *A. cylindrica*=*S. capricornutum* > *S. obliquus* > medium *D. magna*=*P. bursaria*=pH > large amphipods=*Lyngbya*
81	pH > nitrate=small amphipods=turbidity > *S. obliquus*=small D. magna > *A. falcatus* > *C. vulgaris*=*Lyngbya* > medium *D. magna*
84	pH > *A. falcatus*=small *D. magna* > large amphipods=*S. obliquus*=turbidity > small amphipods > medium *D. magna* > *P. bursaria*=*S. capricornutum*
88	pH=small *D. magna* > medium *D. magna* > nitrate > *C. vulgaris*=turbidity > *P. bursaria*=*S. obliquus*=small amphipods > DO
91	Ostracods=pH > small *D. magna* > *S. obliquus* > *P. bursaria*=turbidity > small amphipods > DO > *C. reinhardi* > *A. falcatus*

[1]Variables listed have a quality of 0.5 or greater.
[2]Equals sign between variables denotes equal rank.
[3]Greater than sign between variables denotes greater rank of the first variable.

Table 4: Important variables for four clusters in the NCAA analysis[1]

Day	Variables in rank order
11	Large *D. magna*=small *D. magna*[2,3] > DO > pH > medium *D. magna*
14	DO > medium *D. magna* > large *D. magna*=small *D. magna* > pH=turbidity > *S. capricornutum* > *S. obliquus*
18	Medium *D. magna* > small *D. magna* > *S. capricornutum* > pH=DO > nitrate > large *D. magna* > *C. reinhardi*
21	DO > *S. obliquus* > *P. bursaria* > small *D. magna* > medium *D. magna*=*A. falcatus* > pH
25	pH > DO > *S. capricornutum* > phosphate > *S. obliquus* > small amphipods > small *D. magna* > *Stigeoclonium* > turbidity
28	Small *D. magna* > pH > *S. obliquus* > turbidity > DO > *S. capricornutum*
32	pH > phosphate > DO > *S. obliquus* > *S. capricornutum* > turbidity > large amphipods > medium *D. magna*
35	DO > pH=*S. obliquus* > turbidity > small amphipods > *C. reinhardi* > *Stigeoclonium* > *A. cylindrica*=ostracods

39	*S. obliquus* > medium *D. magna*=small *D. magna* > DO > phosphate > *A. cylindrica*=medium amphipods > *Philodina*
42	DO > small *D. magna* > *A. cylindrica* > *S. obliquus* > turbidity > pH > medium *D. magna*
46	Small *D. magna* > pH > DO > *S. obliquus* > phosphate > *A. cylindrica* > *Lyngbya*=medium *D. magna*=turbidity
49	*A. cylindrica* > DO=pH > medium *D. magna* > *S. obliquus* > small amphipods > *Lyngbya*=small *D. magna*
53	DO > *S. obliquus* > pH > phosphate > A. cylindrica > *P. bursaria* > medium *D. magna* > *A. falcatus* > small *D. magna*
56	pH > medium *D. magna* > *A. cylindrica* > DO > small *D. magna* > *C. vulgaris* > *P. bursaria*=*Philodina*
60	DO > pH > *P. bursaria* > *S. obliquus* > *A. cylindrica* > medium *D. magna* > *A. falcatus* > phosphate > ephippium
63	DO=pH > small *D. magna* > *P. bursaria*=*S. obliquus* > *A. cylindrica*
67	*S. obliquus* > *P. bursaria* > *A. cylindrica* > phosphate > *C. vulgaris*=small *D. magna* > ephippium > DO
70	*P. bursaria* > small *D. magna* > DO=medium *D. magna* > small amphipods=turbidity > *S. obliquus*
74	Medium *D. magna*=small *D. magna* > DO > ephippium=*S.capricornutum* > *P. bursaria*
77	*P. bursaria* > *A. cylindrica* > DO=large *D. magna*=*S. capricornutum* > small *D. magna*
81	*S. obliquus* > small amphipods > *P. bursaria* > nitrate > phosphate > DO > *C. vulgaris* > *Philodina* > *A. falcatus*
84	*S. obliquus* > turbidity > pH > small amphipods > DO > *P. bursaria* > *A. falcatus* > ephippium
88	*S. obliquus* > DO > turbidity > *A. falcatus*=*Lyngbya*=nitrate > *P. bursaria*=pH > small *D. magna*
91	*P. bursaria* > *A. falcatus* > small *D. magna*=turbidity > ephippium > *S. obliquus* > DO

[1]Variables listed have a quality of 0.5 or greater.
[2]Equals sign between variables denotes equal rank.
[3]Greater than sign between variables denotes greater rank of the first variable.

Space-TimeWorms

Using some of the variables shown to be important in the clustering analysis, dynamics were evaluated using space-time worm projections. Using *Philodina* and *A. falcatus* populations, two groups were apparent throughout the length of the experiment (**Figure** 24). However the differences were primarily the result of only one of the variables at any given time. A dramatic difference in *A. falcatus* populations can be seen just after dosing with the jet fuel where the JP-8 and JP-8/heat treatments show an algal bloom. *Philodina* populations also show a distinction between JP-8 dosed and nondosed treatments just after the heat shock. Near the end of the experiment, *A. falcatus* populations again appear to be showing treatment related differences with a hint of a distinction between all four groups at this point.

The space-time worm projection using *A. falcatus* and *P. bursaria* populations again shows two groups just after dosing with jet fuel based on *A. falcatus* populations. This is followed by a long convergence period with little variance within treatments through about day 63. At this point, marked by the torus, the treatments become distinct from each other based upon *P. bursaria* populations. The JP-8/heat only treatment shows the greatest number of *P. bursaria* and the greatest variance within a treatment (**Figure** 25).
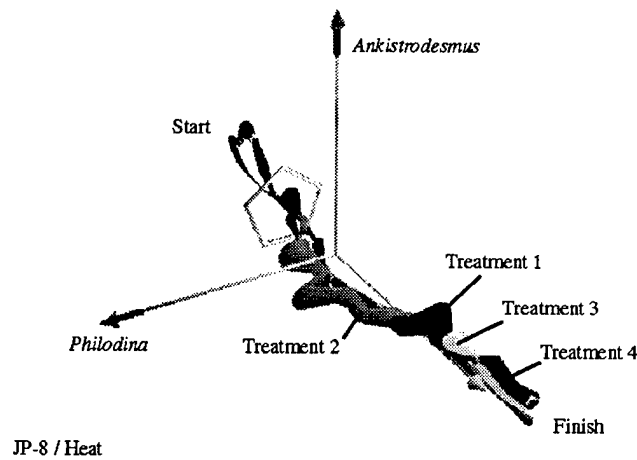
Figure 24. Space-time worm projection of *A. falcatus* and P*hilodina* populations. Two groups are distinct after dosing with JP-8 showing the algal bloom in the treated microcosms [JP-8 (yellow) and JP-8/heat (red)]. *Philodina* populations also show a dose effect with the reference (blue) and heat only (green) treatments showing similar dynamics as do the JP-8 dosed treatments. The torus marks day 35.
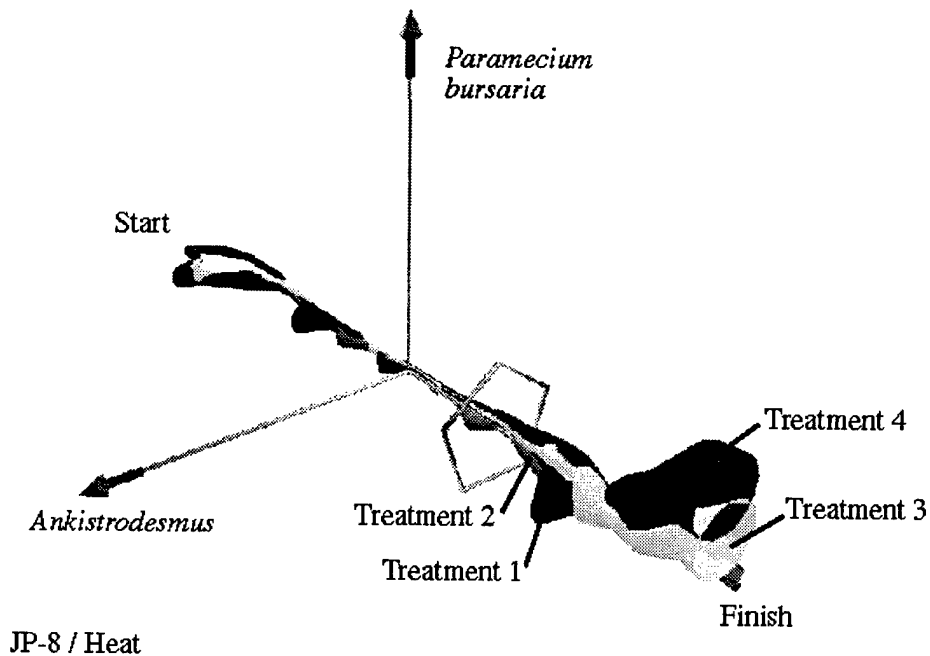
Figure 25. Space-time worm projection of P. bursaria and A. falcatus populations. P. bursaria populations exhibit distinct treatment related responses at the end of the test period. The torus marks day 63. [Reference (blue), Heat only (green), JP-8 only (yellow), and JP-8/heat (red)]

Looking at small amphipod and *A. cylindrica* populations, no striking differences are apparent until after approximately 30 days when *A. cylindrica* populations begin to show an elevation in the quantity of algae in the JP-8 dosed groups as compared to the nondosed groups. There also appears to be some distinction between the JP-8 and JP-8/heat treatments. After a convergence period, small amphipod populations begin to show strong treatment related differences about day 60. By the end of the experiment, there are four distinct treatment groups. The heat only treatment shows increased numbers of small amphipods in relation to the reference treatment and the JP-8 dosed treatments (**Figure** 26).
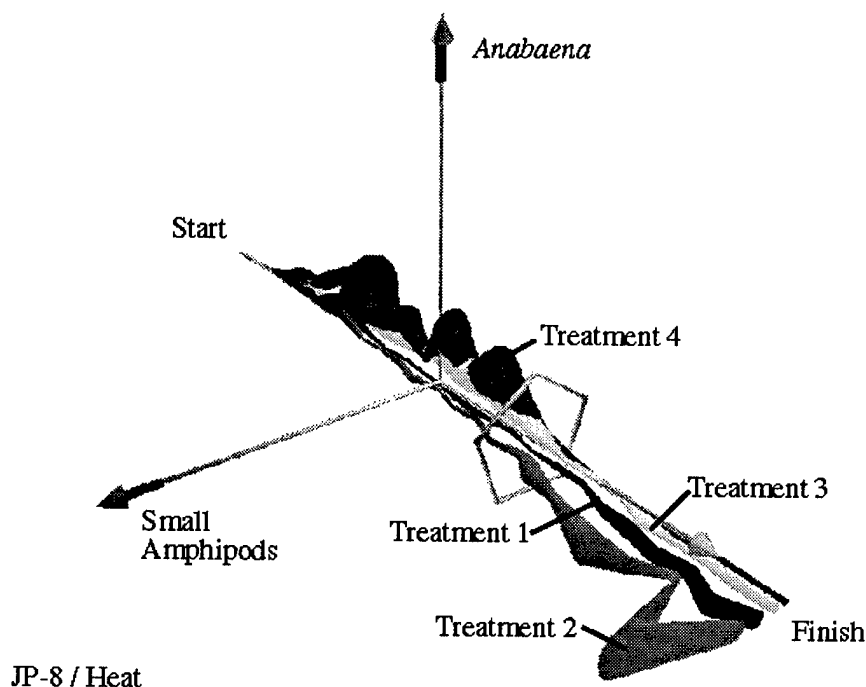
Figure 26. Space-time worm projection of *A. cylindrica* and small amphipods. *A. cylindrica* shows treatment related effects shortly after dosing with JP-8 with greater quantities of algae in the treated microcosms. This pattern persists until day 63 (marked by the torus). At this point, small amphipod populations begin to show distinguishing dynamics. By day 91, the amphipod populations are distinct in each treatment. [Reference (blue), Heat only (green), JP-8 only (yellow), and JP-8/heat (red)]

JP-8/JP-8 Results

The results from the JP-8/JP-8 experiments are in the final stages of analysis and the highlights are reviewed. As in all of the experiments, a differentiation based on treatment is observed during the initial stages (**Figure 27**). The clusters were comprised of those treatments that did not receive JP-8 on day 7 (treatments 1 and 2) and those that did (treatments 3 and 4) The pattern was transitory and did not remain constant from day 33 to 63 of the experiment. A second dosing occurred on day 63, treatments 2 and 3 were dosed and treatments 1 and 4 did not receive the toxicant. This pattern of treatment was identifiable until the end of the experiment.

JP-8/JP-8 NCAA Results
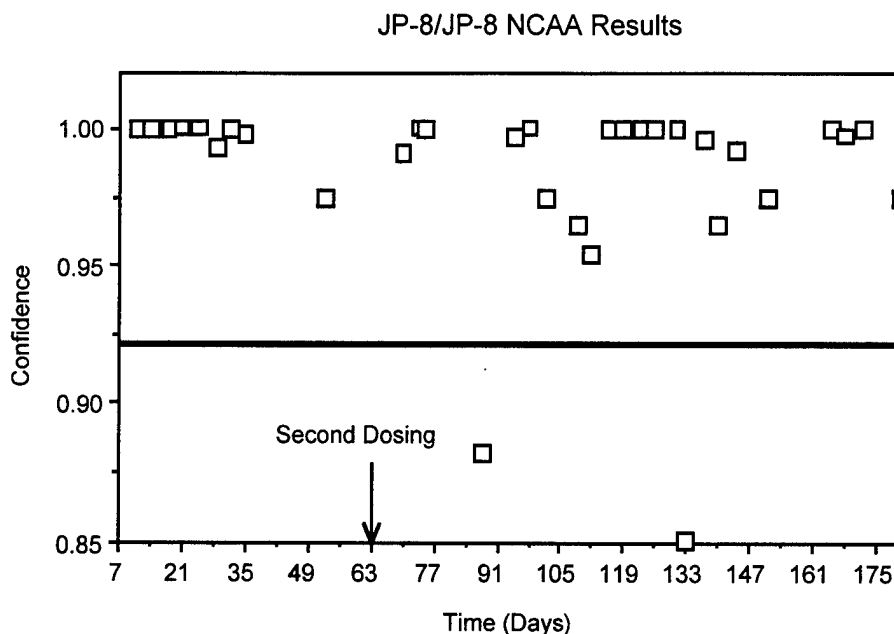
Figure 27. NCAA results for the JP-8/JP-8 experiment. In the initial stages of the experiment the clustering (2 groups) was highly significant with good cluster quality. The pattern was not apparent from day 35 until after the second dosing on day 63. After day 63 the clustering was significant and with good quality until the end of the experiment.

Of the variables measured, pH was the most important in identifying clusters throughout the microcosm experiment. Figure 28 demonstrates the relationships between the patterns seen in the pH and using the NCAA clustering. The initial differentiation of the treatment groups is clearly seen during the first 30 days of the experiment. This difference in pH is associated with the clustering as identified by the NCAA. As the significant clustering, indicated by the shading, is present so is an identifiable difference in pH. When the clustering is not significant, no clear pattern in pH can be observed. After the second dosing the clustering is re-established and a clear difference in pH can be observed. The clustering is not significant for a period but then is re-established as is another, but different pattern in the pH measurements. As indicated by the shading, on one sampling day the clustering pattern reflected the pattern as seen due to the original dosing. This may be a chance event or perhaps does reflect some aspect of the original pattern.

Although pH was a critical value, when combined with Small Daphnia, other patterns emerged. **Figure 29** presents a Space-Time Worm incorporating the variable Small Daphnia along with pH during the fist half of the experiment. A change in the daphnid populations can be clearly seen early in the experiment. The corresponds to the toxicity of the WSF to the daphnia, and an algal bloom results. The change in algal metabolism is identified by the alterations in pH. In the central section of this graph the four treatment groups are indistinguishable. This corresponds to the lack of clustering observed by the NCAA. At the end of the experiment a new pattern emerges dominated by the pH with the Small Daphnia not as significant a contributor to the differentiation.
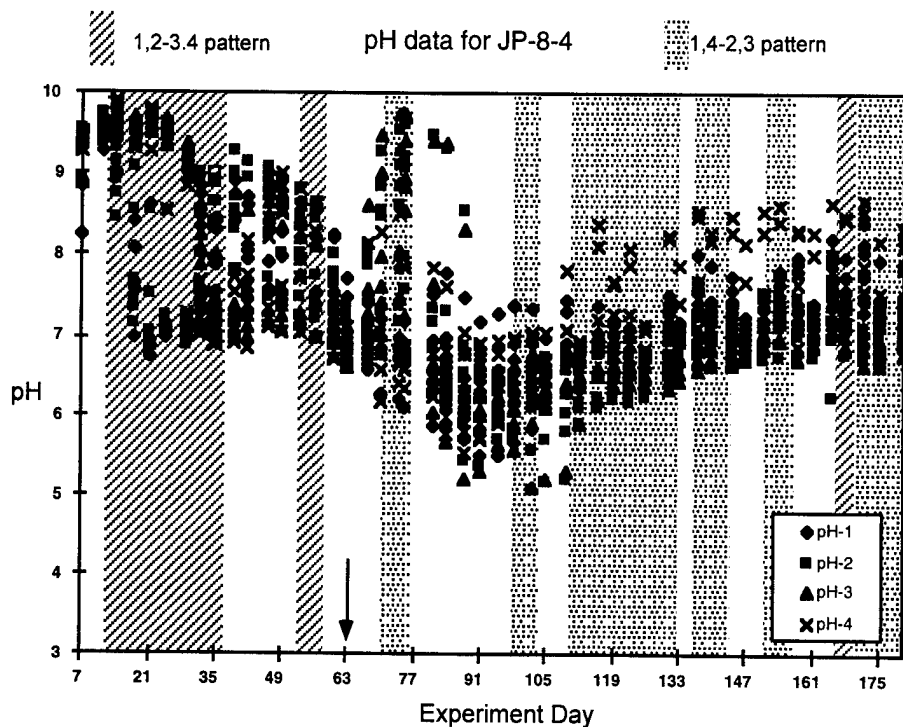
Figure 28. NCAA results combined with pH and cluster type. Each pH value is plotted as a point and the shading corresponds to significant clustering and to the type of clustering. During the early period of the experiment, two groups are identifiable by pH and these correspond to the groups identified by the NCAA. A pattern did not reappear consistently until after the second dosing on day 63. The treatment groups dosed on day 63 became another pattern seen in the pH data and also in the clustering until the end of the experiment. Note that two pH patterns are apparent after the second dosing, between days 70 and 77 and from day 110 until the end of the experiment. Additionally, a clustering that reflected the original dosing on day 7 was observed on day 168.
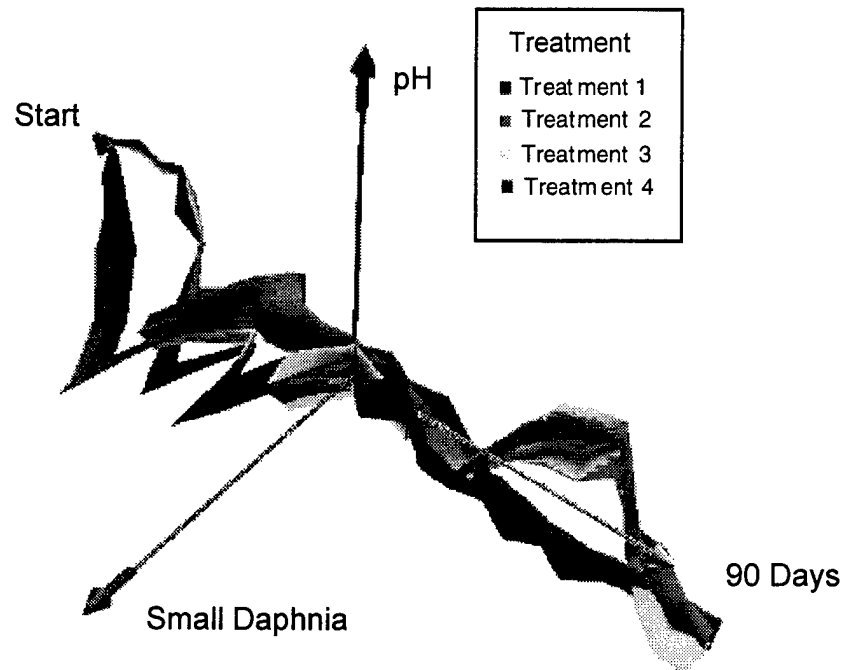
Figure 29. Space-Time Worm for the first 90 days of the JP-8/JP-8 experiment. The initial differentiation in the early part of the experiment is clearly visible. the second differentiation shows that the treatments have set new pairs corresponding to the dosing pattern on day 63.

The last 90 days of the experiment are presented in **Figure 30**. Using the combination of the Small Daphnia and pH axes, the persistent differentiation in the treatment groups is clear. Treatment 2 and 3, dosed on day 63, remain together as do Treatments 1 and 4. Only on one sampling day do the two sets of trajectories intersect near the end of the experiment. This intersection corresponds with a significant clustering, but that clustering was representative of the treatment on day 7.
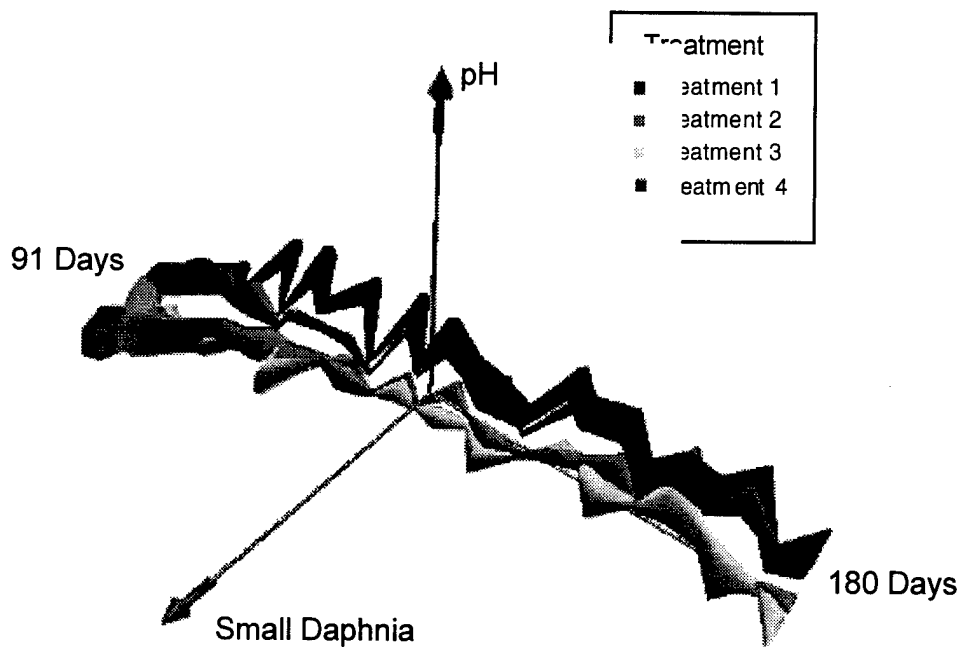
Figure 30. Space-Time Worm for day 91 day to day 180 of the JP-8/JP-8 experiment. The two groups associated with the dosing pattern of day 63 are maintained during the remainder of the experiment.

Discussion

In both sets of experiments the persistence of effects after the demise of the stressor was observed. The principle foundation of community conditioning, the persistence of effects, was confirmed in both experiments.

After the heat stress, NCAA significance values, cosine distance measures the dynamics of *P. bursaria*, amphipod, and *S. obliquus* populations suggested that additional groups were present. However, strong patterns of differentiation were not seen. The heat shock was a more mild stressor; thus, while more than two groups may exist, they are very likely subsets of the two strong clusters associated with jet fuel as detected by the statistical analyses. Further investigation into this data set is merited.

Indirect effects played a significant role in elucidating the treatment related responses that support community conditioning. Many population level responses indicated that the jet fuel treatment had lasting effects that became apparent long after the stressor had left the system. Similarly, the differences in the populations of *P. bursaria*, amphipods, and *S. obliquus* that suggested more than two groups also occurred after the heat stress was gone from the system. These indirect effects would have been missed had the experiment been terminated at day 63, the standard length of the SAM.

Support for Community Conditioning

As with previous microcosm studies (Landis et al. 1993, 1994), the univariate statistical analyses provided little unexpected information about the dynamics in the microcosms. There were significant treatment related responses in water chemistry, algal populations, and daphnid populations just after the dosing with the WSF of JP-8 on day 7 and continuing through day 32. This is not surprising considering the acute toxicity of the jet fuel to *D. magna*. With the *D.*

magna gone or significantly reduced in numbers, the resulting algal bloom in the fuel treated microcosms created changes in the water chemistry, raising the pH, DO and turbidity.

While effects of the JP-8 stressor were evident in univariate statistical analyses, the effects of the heat shock were not. When differences were detected for individual taxa and water chemistry after the heat shock (day 33), multiple comparison tests primarily identified significant differences between groups that were treated with JP-8 and nondosed groups. However, as indicated previously, responses after day 33 were often scattered across the test period and did not follow a consistent or coherent pattern. When univariate statistics did indicate differences between JP-8 and JP-8/heat treatments or the reference and heat only treatments, there was no consistent pattern to these differences, either.

When variables such as *P. bursaria, S. obliquus*, and amphipods appeared to demonstrate treatment related responses after the heat shock, univariate statistics did not detect significant differences related to the heat stressor with the exception of the differences in small and medium amphipods populations. For the *P. bursaria* populations, multiple comparison tests only detected differences between the JP-8/heat and reference treatments on a consistent basis even though *P. bursaria* were designated as important by NCAA for four clusters and appear so distinct graphically (**Figure 11**) and in the space-time worm projection (**Figure 25**). Similarly, *S. obliquus* was chosen as an important variable for four clusters in NCAA and **Figure 4** shows distinct differences between *S. obliquus* population means in the JP-8 and JP-8/heat treatments. However, Tukey's test did not find these differences statistically significant.

Likewise, small amphipod populations exhibited a strikingly distinct treatment related response both graphically (**Figure 9**) and in the space time worm projection (**Figure 26**). Yet, univariate statistics did not indicate that four distinct treatments were present. The heat only treatment was different from all other treatments and the reference treatment was different from the JP-8/Heattreatment.

Thus, the combined univariate results indicate the presence of just two groups and with the exception of the amphipod populations, these groups follow the jet fuel/no jet fuel dichotomy. Accordingly, univariate analyses detected only one strong divergence away from the reference treatment. This oscillation occurred from day 11 to 32 and was caused by the toxicity of the jet fuel. Based upon the results for most variables, the reference and heat only treatments displayed similar dynamics as did the JP-8 and JP-8/heat treatments. Thus, univariate methods were successful in identifying the two treatments present after jet fuel dosing. While some variables demonstrated differences between dosed and nondosed groups after day 32, the lack of consistency in most of these differences makes it difficult to identify any changes in community dynamics after this point in the experiment based upon the individual components of the system.

NCAA results, however, detected a lengthy divergence in community dynamics for the entire test period. This divergence is very strong just after the JP-8 dosing, showing two distinct groups in both the clustering quality and the association analysis (**Figure 19**). These two groups consist of the reference and heat only treatments and the JP-8 and JP-8/heat treatments. The association for two clusters remains statistically significant for most of the sampling dates. There is a brief period of decreased significance near the end of the test period but the association for two clusters again becomes very strong from days 81 to 91 (>.99). The periods of divergence and the oscillations indicated above are strong patterns occurring after day 32 that were not clearly identified with ANOVA or the multiple comparison tests.

While univariate statistics did not identify more than two groups during the experiment, multivariate analyses did indicate the presence of additional groups. Using NCAA, a weaker clustering quality is evident for four clusters just after dosing with JP-8 but cluster quality after the heat shock is very similar to that for two clusters (**Figure 21**). Moreover, the association of clusters with the treatments remains significant and similar in magnitude to that of two clusters throughout most of the test period (**Figure 22**). As with two clusters, there is a brief period of decreased significance near the end of the test period. However, the association for four clusters again becomes very strong from days 84 to 91 (>95%). Results from the metric clustering analyses support NCAA and the presence of four groups at the end of the test. The

cosine analysis in particular shows a significant association for most of the test period (**Figure 13**). However, metric clustering, like NCAA, detected four clusters prior to the heat shock where only two groups were expected.

Two or Four Clusters?

Univariate and multivariate techniques strongly support the presence of two groups from days 11 to 32. Furthermore, the multivariate results presented above indicate that two groups are statistically significant for the length of the experiment. Yet, multivariate techniques also revealed a significant association for four groups throughout much of the experiment. While two groups are strongly supported by other results throughout the test period, there is no strong evidence to support the presence of four groups at any time in the experiment.

For days 11 to 32, a significant four-clustering is not expected but the association analysis in both NCAA and metric analyses is greater than 95% on these days. In the NCAA and metric association analyses, when two strong clusters are present, a statistically significant four-clustering will automatically result when there are only two real groups. Also, a significant result can occur as a result of the chi-square analysis used by NCAA. With only 24 points to classify and the presence of two strong clusters, it is not difficult to get a near correct classification for four treatments by chance. A good classification in any one group would drive the significance level to a higher than expected value.

The contingency tables and important variables identified in the NCAA analysis provide some help in interpreting the results from days 11 to 32. These results suggest that two rather than four clusters are really present. Results from two clusters show correct assignment to treatment group for 23 of 24 microcosms on all but day 28 when the assignment was perfect. This indicates a strong clustering and is supported by the very high cluster quality values for two clusters during this part of the test. In contrast, the assignment of microcosms to treatment groups with four clusters is much less accurate, for instance, 15 of 24 correctly assigned. Accordingly, the cluster quality values for four clusters during this period are much lower than those for two clusters. Notably, the misclassifications for four clusters for days 11 to 28 occur within the jet fuel treated or untreated groups indicating the difficulty in distinguishing between microcosms within these subgroups.

Similarly, examining the distance tables for the metric analyses also suggests two distinctive groups even though the significance levels are greater than 95%. This is also shown graphically in the plot of the cosine distances relative to the reference group (**Figure 15**), particularly through day 18.

Moreover, the variables identified as important by NCAA (**Tables 3** and **4**) for days 11 to 32 for two or four clusters were those that only exhibited clear differences, both graphically and in ANOVA, between treatments that received jet fuel and those that did not. Thus, even though similar significance values were obtained for two and four clusters, the weight of evidence points toward two clusters between days 11 and 32 and suggests that the presence of four clusters is not meaningful.

In contrast to four clusters prior to the heat shock, the presence of two strong clusters in the NCAA and metric analyses after the heat stress is more easily explained. The jet fuel was a very strong toxicant with immediate lethal effects on some organisms whereas the heat shock was a relatively mild stressor likely exhibiting effects on the molecular rather than the population level. Thus, the persistence of two clusters throughout the experiment is appropriate and these clusters are strongly associated with the JP-8 treated or untreated microcosms. While the contingency tables do show that the accuracy of assignment to treatment group with only two clusters decreases by day 56, this accuracy does improve to near perfect after day 77.

Meanwhile, multivariate analyses also suggest four groups after the heat shock when there are technically four treatment groups. These clusters are identified as statistically significant by both NCAA and metric analyses for much of this time. However assignment to treatment group was not as expected for four clear groups in the NCAA analysis. Examining the contingency tables, the best assignment was 17 of 24 microcosms. Misclassifications often occurred between JP-8 treated groups or untreated groups, particularly from days 46-56 and 84-91,

4-33

indicating that these subgroups are still similar to one another. This is supported by the lingering presence of two strong clusters after the heat shock. Yet during this time, the cluster quality for two and four clusters is very similar unlike the large differences seen just after dosing with JP-8. These results suggest that four clusters may be present but are more difficult to define than two clusters because of the weaker nature of the heat stress. This would help explain the misclassification in the contingency tables.

In addition to multivariate results, there is also evidence to suggest the presence of more than two groups in some of the organism population dynamics. Small and medium amphipod populations in the heat only treatment were consistently statistically different from the other groups after the heat stress and small amphipods populations in all groups appeared different graphically and in the space-time worm projections (**Figures 9** and **26**). Additionally, S. obliquus populations in the JP-8 and JP-8/heat treatments appear very distinct from each other and from the reference and heat only treatments from day 42 to 77 (**Figure 4**). Furthermore, P. bursaria populations consistently appear very different in all groups graphically and in the space-time worm projections (**Figures 11** and **25**). However, with the exception of the small amphipod populations, these results were not statistically significant outside of the jet fuel/no jet fuel dichotomy. While the selection of these particular variables for detecting four treatments in the four-cluster NCAA analysis (**Table 4**) also supports the presence of four clusters, it does not provide conclusive evidence. This does not mean that the information imparted by the heat stress is not maintained within the systems, only that it could not be convincingly detected with the available analysis methods.

The more mild nature of the heat stress and the presence of only three or four variables that suggest differences between all treatments helps explain the lack of significance in the univariate analyses and the difficulty of correct assignment in the NCAA contingency tables for four clusters. While more than two groups may exist, they are very likely subsets of the two strong clusters detected by the statistical analyses. The close proximity of the subsets to each other in multivariate space would make obtaining a distinct differentiation more difficult (**Figure 27**). Further investigation into this data set is warranted.

Perhaps the responses of the amphipod, P. bursaria, and S. obliquus populations would have been stronger after day 91 or perhaps additional variables would have exhibited four distinct responses had the experiment been extended past 91 days. Regardless, because strong patterns of differentiation were not seen for four groups, the only clear conclusion that may be drawn from the results is that at least two groups are present for the entire course of the experiment. Community conditioning is still supported by this conclusion as recovery did not occur during the experiment.
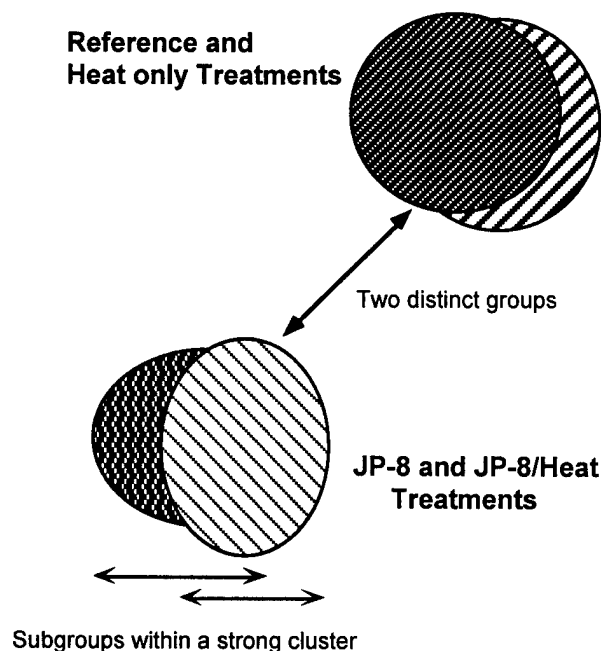
Figure 31. Representation of the overlap among treatments dosed with JP-8 and among those that were not dosed with jet fuel.

Community Dynamics

In addition to demonstrating the presence of treatment associated clusters during the experiment, NCAA also provided a ranking of the important variables in determining the clusters. These variables changed over the course of the experiment as the communities themselves changed. The strong divergence between the JP-8 dosed and nondosed treatments resulted from changes in the predator/prey interactions in the treated microcosms. The producers, primarily the unicellular algae, were freed from the constraints of the primary consumers (*D. magna*) in the treated microcosms and an algal bloom resulted. Appropriately, the variables listed as important by NCAA during the first 4 weeks of the test were the unicellular green algae that the daphnids preferentially feed upon, *D. magna*, and water chemistry.

However, after day 32 blue-green algae began to become important. By this time in the experiment, the systems were nitrate and phosphorus limited. *A. cylindrica* are able to fix nitrogen and utilize phosphorus at very low levels relative to other algal species. This probably enabled the blue-green *A. cylindrica* to increase in numbers relative to other algal species as seen in **Figure 6**. The increase in quantities of *A. cylindrica* was more apparent in the JP-8 dosed groups, probably because they became phosphorus limited before the nondosed treatments which would give *A. cylindrica* a competitive advantage.

Similarly, the quantities of *S. obliquus* in the JP-8 treated or untreated microcosms were very different. Treated microcosms exhibited increased quantities of the algae for the length of the experiment. The lack of predation in the JP-8 and JP-8/heat treatments after dosing with WSF enabled *S. obliquus* populations to increase significantly (**Figure 4**). As nutrients were depleted and as the *D. magna* populations began to rebound in treated systems, the populations of *S. obliquus* did decrease, but quantities remained statistically higher than in the nondosed microcosms. Because *S. obliquus* exists in colonies of four cells, it's size makes it less available to *D. magna* as a food source (ASTM E 1366 1991). This size factor coupled with the initial freedom from predatory constraints could explain why quantities of *S. obliquus* remain greater than that of the other unicellular algae from day 25 to 70.

Factors such as differences in *D. magna* and algae populations also created changes in the detritus of the microcosms. Differences in detrital quality were very apparent. For several sampling days after dosing with JP-8, microcosms treated with jet fuel had a lush green algal mat on top of the sediment while the nondosed treatments did not. As the experiment continued, the reference and heat only treatments developed a detritus base that consisted primarily of consumed algae. Meanwhile, detritus in the JP-8 treated groups consisted of dead algae and, to a lesser extent, consumed algae since the *D. magna* populations had only recently rebounded.

The amphipods, being detritivores, became more prevalent in all treatments as a detrital base formed but showed reduced numbers in the treatments dosed with jet fuel. This may be an indication of jet fuel toxicity, preference for particular detrital quality, or both. There were also distinct graphical differences between all populations of amphipods at the end of the test period. Amphipod populations in the heat only treatment displayed increased numbers in relation to the other treatments suggesting that the heat shock somehow altered the system in such a way as to favor the amphipods. Yet in the JP-8/heat treatment, amphipod numbers were reduced relative to all other treatments indicating that the combination of heat and jet fuel was detrimental. Whether this was a direct or indirect effect is unknown.

During the latter third of the experiment, the biotic components of the system drive the clustering. *D. magna* are still important and this is not surprising since they exert such a strong influence on the system through their efficient grazing. However, the detritivores and bacteriovores are frequently identified as important at this stage (Tables 3 and 4). These components are also the ones that demonstrate the most distinct treatment related differences. This suggests that the unmeasured detritus and microbial components of the system may indirectly hold the information imparted to the systems by the stressors.

Importance of Indirect Effects

In addition to demonstrating the presence of community conditioning, the JP-8/heat shock microcosm experiment also clearly showed the importance of indirect effects on aquatic communities. The jet fuel stressor occurred on day 7 and was most likely gone from the system by day 28. The lack of significant differences by day 32 for most variables as detected by ANOVA would seem to support this. The heat shock occurred on day 33 and after this point, the systems were subjected to no other stressors.

Yet, some of the most distinct differences in dynamics were clearly visible in the systems long after day 33. These were the indirect effects propagated by the direct effects that occurred at the time of the stressors. However, these effects were not visible until later in the test period when the effects were either great enough to detect or not obscured by the direct effects. The indirect effects that surfaced near the end of the 91-day test period were those that enabled detection two groups for the length of the experiment. Indirect effects were also those that suggested that more than two groups might be present.

Some variables appeared to express the indirect effects of both the jet fuel and the heat shock. *P. bursaria*, *S. obliquus,* and amphipod populations are examples of this but only the latter was statistically significant. Other effects appear to result from the chemical stressor JP-8. This is seen in the *D. magna* populations and the space-time worm projection of *A. falcatus* and *Philodina* populations.

Additionally, the reduction in ephippium production in the JP-8 and JP-8/heat treatments relative the reference treatment may also be viewed as an indirect effect. Ephippia are sexual daphnid eggs and their production is associated with overwintering or with stress (Goulden and Henry 1990). Even though laboratory daphnids are not exposed to the environmental changes associated with seasons, they have been known to produce ephippia in the fall in the absence of stress. The JP-8/heat microcosm took place in the fall, thus, ephippium production in the reference group was likely associated with the overwintering preparation. The reduction in ephippium production in the JP-8 and JP-8/heat treatments suggests that the stressors in this experiment may have interfered with the ability of *D. magna* to respond appropriately to seasonal signals.

If the experiment had ended on day 63 as specified in the protocol, many of these dynamics would have been missed. Had this occurred, systems would have been seen as becoming very similar to one another, an incorrect conclusion. These indirect effects question the idea that systems can ever return to an original state.

Additionally, the heat stress was a weaker stressor designed to merely nudge the systems. Even so, the heat stress propagated effects in the amphipod populations that are seen long after the heat shock itself. Thus, a stressor does not have to be severe to leave lasting effects.

Of course, these are just the effects on the variables that were measured. As mentioned previously, the detrital quality and microbial components of the systems are not quantified in the current protocol. Based upon observations during the experiment, detrital quality could provide significant insight into the effects of stressors on aquatic systems.

Also rarely considered in tests like this are the changes in the genetic compositions of the affected populations. When a large number of individuals are killed in a stress event, genes are lost from the gene pool. Changes in the mitochondrial DNA composition of the population may occur and events such as founder effect may also result. Changes in the genetic composition of a population may alter the response of a population to a subsequent stressor or reduce the fitness of the population. Such changes would be indirect effects that may not produce visible changes in community dynamics for many generations.

Thus, while indirect effects may be difficult to detect, they are likely present and maintain the ability to alter the course of an ecological system, preventing it from returning to an original state. This has been demonstrated in this experiment and in previous microcosm experiments (Landis et al. 1993a, 1994). Thus, indirect effects carry information imparted to a system by a stressor and are, therefore, an important aspect of community conditioning.

Conclusions

The JP-8/heat microcosm supports community conditioning: treatments dosed with jet fuel displayed different patterns over time compared to those not dosed with the fuel, even after the cessation of the stressor. While univariate methods did not detect differences for most variables after day 32, AI techniques clearly demonstrated that at least two groups were distinguishable throughout the experiment. Thus, even these simple ecological systems are historical and irreversible, retaining the information imparted to them by the jet fuel. The treatments did not converge back to the reference state.

While there was no conclusive evidence that the heat stress had a detectable effect on systems, there was some suggestion that the information imparted by the heat shock was maintained by the system. This suggests that past stressors can affect responses to future disturbances and, if supported by additional investigation into the data set, would further support communityconditioning.

Results also show that indirect effects are very important and often linger. In the present study, the indirect effects made the detection of differences between treatments possible after day 32. *P. bursaria, S. obliquus,* amphipod populations and pH demonstrated the most obvious indirect effects throughout the experiment. For the amphipod populations, this distinction included the effects of the heat stress.

Consequently, the results force a reevaluation of the assessment of ecological systems. As this and previous microcosm experiments demonstrate, ecological systems maintain information imparted to them by disturbances. This information may include all of the disturbances in the history of the system. But, the variables that are important in defining a system change over time. Unfortunately, processes such as risk assessment and many ecological studies often rely on a preselected set of variables and on indices that condense data to determine the probable or actual impact to an area. The results from many microcosm studies suggest that sampling a broader array of variables and relying on AI techniques such as NCAA can help eliminate these problems while providing information not available with conventional analysis methods (Landis et al. 1994).

It also appears that the concepts of stability and recovery as they apply to ecological systems are not supported. As such, new ways of studying and defining these systems

becomes necessary. Because most if not all disturbances will affect the trajectory of a system, questions that are grounded in the expectation that a system will recover are inappropriate and instead should ask where will the system go from here. However, while predicting changes in the short term may be possible, community conditioning suggests that forecasting is limited because everything cannot be measured and what is not measured could contain the information that will alter the future trajectory of the system.

Therefore, determination of acceptable effects and realization that systems will change over time becomes necessary. This means changing the current approach to ecosystem management and assessment. Adhering to false paradigms can only hinder our ability to better understand how systems respond to disturbance.

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Section 5: Metapopulation Dynamics: Indirect Effects and Multiple Discrete Outcomes in Ecological Risk Assessment

Abstract—A toxicant-dosed metapopulation model was used to explore the range of possible dynamics of populations in contaminated field sites. A single species metapopulation model was developed that is discrete, deterministic, and incorporates dose response curves and biotic growth rates to describe the effects of contamination on a metapopulation. The distribution of the chemical contamination is assumed limited to one patch and contagious within that patch. Both persistent and degradable toxicants were modeled. Five principal conclusions resulted from our simulation studies: (1) Mortality in one subpopulation has ecologically significant effects on nondosed subpopulations. This hypothesis we term "action-at-a-distance," because no direct contact with a toxicant has occurred except in the dosed patch. (2) Because uncontaminated sites connected to the contaminated site by migration of the biota are affected, these uncontaminated sites cannot be reference sites. (3) The arrangement of the patches is critical to the dynamics of the system and the overall impact of a toxicant. (4) Due to the contagious distribution of the toxicant and the stochastic function describing exposure-effect, multiple discrete outcomes often are possible from the same initial conditions. These outcomes can range from extinction to the reaching of the carrying capacity for a patch. (5) If sufficient cleanup is not possible, it may be necessary to isolate the contaminated patch, allowing the formerly connected patches to regain more typical population dynamics.

Introduction

Neither toxicants nor organisms are uniformly distributed in time and space. To deal with this in an experimental fashion, we must formulate a baseline construct. Such a construct should be consistent with what is currently understood regarding the dynamics of community structure, the interactions of conspecific populations, and the distribution of toxicants in the environment. We have incorporated the basics of community conditioning, the patchy distribution of organisms, metapopulation dynamics, and the heterogeneous distribution of chemical toxicants into a construct to describe the impacts of toxicants. The goal is to develop specific questions so that experiments can be designed to discover the rules that govern impacts in a patchy ecological environment.

Distribution of organisms and toxicants

Organisms tend to exist in highly clumped (contagious) distributions as opposed to Gaussian or regular distributions. Evans [1] demonstrated using experimental evidence that plant and insect populations typically are clumped (contagious) in nature. Plant data from a variety of British and North American localities were found to fit a Neyman Type A distribution. Insects were distributed as a negative binomial. Taylor [2] demonstrated that the relationship of the mean of a population density is generally related to the variance by a simple power law: Variance is proportional to a fractional power of the mean. This relationship was confirmed in the examination of 23 animal data sets including worms, insects, marine invertebrates, and spiders. In each data set, the distribution became more clumped at higher densities, and at lower densities the aggregation diminished. Recent research by Taylor and his associates has demonstrated the spatial and temporal dynamics of patches [3,4], especially in insect populations.

Landis [5,6] observed that populations of the ciliate protozoa of the species complexes *Paramecium aurelia* and *P. bursaria* exist in aggregate patterns characteristic of the species. The pattern of distribution of paramecium can be tied to life history strategies and population genetics [7-9].

Wu and Loucks [10] incorporated the dynamic heterogeneity of ecological systems into a hierarchical patch dynamic framework. This framework includes scale, the patchy distribution of organisms in time and space, and stochastic functions when describing patterns in ecological systems. Patchiness in the distribution of organisms is a fundamental property of ecological

systems and must be explicitly incorporated into a description of how toxicants impact such entities.

Both toxicants and the physical-chemical parameters that control exposure exist in a contagious fashion with temporal variability. Clifford et al. [11] clearly mapped the distribution of surficial concentration of dieldrin and demonstrated the clumped and irregular distribution of the toxicant. Using derived bioaccumulation factors and toxicological benchmarks, the risk of direct effects to a variety of organisms was also mapped and found to be clumped. Evans and Huggett [12] showed that the concentration of tributyltin (TBT) varied rapidly over time in Hampton River and Sarah Creek in Chesapeake Bay. This annual variation was superimposed over a gradual linear decrease in the TBT concentration.

Temporal and spatial variation exists in sediment. Analysis of sediment cores can provide an indication of both the temporal and spatial heterogeneity of nondegradable materials within a site. A study by Wiegers [13] sampled a typical freshwater pond known to be contaminated with heavy metals. Fifteen cores were taken, and each 5 cm was analyzed for metals using several extraction techniques. A variance-to-mean ratio of greater than 1 indicated a contagious distribution. The variance-to-mean ratio for the metals studied was generally greater than 1 for chromium, lead, and zinc. Assuming that greater depths were indicative of past conditions, it was also seen that the metal concentrations and the variance-to-mean ratio varied greatly over time. A variance-to-mean ratio as high as 53 for chromium was observed in these sediment cores. Besser, Ingersoll, and Giesy [14] have also demonstrated the large variability of sediment acid-volatile sulfide (AVS) concentration in samples from the Clark Fork River of Montana. AVS is a hypothesized controlling factor in the toxicity of the metals in sediments. The variability of AVS was reflected in the outcome of a number of toxicity tests.

Modeling the effects of temporal and spatial heterogeneity

The evidence above clearly demonstrates that both organisms and environmental toxicants exhibit a great deal of temporal and spatial heterogeneity. This characteristic heterogeneity is purposefully minimized in laboratory and field experiments, whether they are single species acute tests or elaborate multispecies toxicity tests. The reason for minimizing this variability is to generate sufficient statistical power to conduct meaningful statistical analyses with practical numbers of samples and replicates. However, this process of minimization excludes heterogeneity, a fundamental property of real ecological structures.

To construct experimental laboratory and field systems that examine the impacts of the heterogeneous distributions of organisms and toxicants, hypotheses concerning potential impacts need to be generated. Models are one of the tools for hypothesis generation, whether conceptual or mathematical. However models do come with attached caveats.

Oreskes, Shrader-Frechette, and Belitz [15] state that the validation or verification of numerical models of natural (open) systems is not possible. The best that can be done is the confirmation of the model by prediction and observation. The confirmation is only partial, because the observation may have equaled prediction by chance alone. These conclusions have important implications for the use of models in public policy and these are discussed extensively. However, the use of models in the construction of testable hypotheses is not challenged by Oreskes et al. and instead is seen as playing a principal role in the scientific process. Models are seen at their best when used to challenge existing constructions and to generate experiments to differentiate paradigms.

Within this context we have constructed models to describe the impacts of toxicants on populations when both the toxicants and the populations exhibit spatial and temporal heterogeneity. The framework that we have used is that of metapopulation dynamics. Metapopulation dynamics explicitly deal with environmental heterogeneity in the distribution of habitats and organisms.

Introduction to metapopulation dynamics

A metapopulation is a "population of populations" [17] connected through immigration and emigration. The populations have specific constraints. The minimum viable population size (MVP) is the population size below which patch extinction occurs. The carrying capacity (CC) is the

population size that can just be maintained without a tendency to increase or decrease. A subpopulation may serve as a sink if it is below the MVP and drains immigrants. A subpopulation may serve as a source for nearby patches by providing immigrants to them.

The fraction of occupied population sites p in a homogeneous habitat changes over time according to the basic formula of metapopulation models [16]:

$$dp / dt = \text{immigration rate - extinction rate.} \quad (1)$$

If $p = 1$, all population sites are occupied, and if $p = 0$, regional extinction has occurred, thus, $0 \leq p \leq 1$.

Levins' initial model of the dynamics of a single species metapopulation in a heterogeneous environment composed of homogeneous and identical patches is:

$$dp / dt = ip\,(1 - p) - ep. \quad (2)$$

In this initial model, i and e are the probabilities of local immigration and extinction, respectively. Hanski and Gyllenburg [17] added the "rescue effect," the idea that immigrants from surrounding population sites may reduce the probability of local extinction:

$$dp / dt = ip\,(1 - p) - ep\,(1 - p). \quad (3)$$

The extinction rate $[ep\,(1-p)]$ is a quadratic function: when p is small, the extinction rate increases as more sites are occupied, but when p is large ($p > 0.5$), the extinction rate decreases as a result of the rescue effect. In contrast, the extinction rate (ep) always increases in Levins' model. The rescue effect decreases the extinction rate by the quantity of ep^2 and is most important at large values of p [17].

Another class of metapopulation models is the diffusion reaction model. The basic formula of such a model used by Wu, Vankat, and Barlas [18] is:

$$dN_i / dt = N_i f(N_i) + \sum_{j \neq i} [d_{ij}\,(N_j - N_i)] \quad (4)$$

Here, d_{ij} is the migration rate of individuals from patch i to patch j and N_i and N_j are population sizes in the patches i and j. The population growth rate is $f(N)$.

Using these equations in a series of simulation studies, Wu, Vankat, and Barlas [18] demonstrated the importance of patch arrangement, size, and migration paths in the persistence of populations within a landscape. Numerous combinations of patch arrangements were examined to explore different conservation scenarios. A linear arrangement of patches was shown to be more protective of the metapopulation as a whole when one of the subpopulations is below the MVP.

Metapopulation models in environmental toxicology
Metapopulation models have been used to examine the dynamics of populations resulting from pesticide application. Sherratt and Jepson [19] investigated the impacts of pesticides on invertebrates using single species and predator-prey metapopulation models. In the case of the polyphagous predator, persistence of the population in the landscape is enhanced if only a few fields are sprayed, the application rate of the pesticide is low, or the intrinsic toxicity of the pesticide is low. There also appears to be an optimal dispersal rate that maximizes the likelihood of persistence of the predator in a sprayed field. Another important finding was that pesticide application patterns can cause the prey insect population to reach higher densities than would

occur otherwise. Dispersal rates of the predator and the prey are important factors determining the prey population densities.

Maurer and Holt [20] recently used several types of metapopulation models to investigate the importance of migration and other factors in determining the impacts of pesticides. Exposure to the pesticide was assumed to decline geometrically to simulate degradation. An increase in migration rate among patches decreased the persistence of the population. The more toxic the pesticide, the less persistent the population. An increase in the rate of reproduction improved the persistence of the population in the landscape. Further investigation also demonstrated that as more of the patches became contaminated, the persistence of the population decreased by reducing the number of potential sites for colonization.

In this study we adopt the metapopulation models of Wu, Vankat, and Barlas [18] to the examination of the impacts of toxicants on a metapopulation containing three patches of varying arrangements. The simulation models also allow the recognition of a variety of dynamics and rates of population growth. Using a simple stochastic function for exposure to the toxicant, we hypothesize that toxicants can have impacts on populations within patches that do not contain the toxicant. We call this phenomena "action-at-a-distance." We also find that multiple outcomes can occur given the same initial conditions, and that these outcomes range for a population within a patch from reaching the carrying capacity to extinction. The probability of each possible result depends on initial population size, toxicant concentration, and distance between patches. Our results cause us to doubt further the existence of reference or control sites in a field situation. Finally, we propose experiments to test the principal hypotheses of this model.

Methods

The toxicant model was constructed and run using the object-oriented programming environment of Stella II. General adaptations were made to the Wu, Vankat, and Barlas [18] model while constructing the toxicant model. Variables representing organism counts (subpopulations, growth, immigration, etc.) were converted to integers, making the model discrete rather than continuous. The discrete variables ensured a more realistic model of the populations than a continuous model by only handling whole organisms. Wu, Vankat, and Barlas' graphical variables based on trends in field data (per capita net growth rate, habitat available, etc.) and density dependence remained unchanged in the toxicant model.

The toxicant models discussed here are general and do not represent particular chemicals or species, but utilize trends from three types of chemicals and animal population data. All of the models limit the application of the toxicant to a single patch. A Poisson distribution is used to describe the exposure of the organisms to the toxicant within the patch.

Model assumptions:

As with any model, a number of assumptions are required. The primary metapopulation assumptions are:
1. The local populations have density-dependent per capita growth rates.
2. Immigration and emigration rates are dependent on the density and the distance.
3. There is no outside dispersal pool.
4. Thresholds such as carrying capacity and minimal viable population do exist and do not change over time.
5. Resources in a patch are not eliminated.
6. No avoidance behavior or compensatory reproduction is exhibited by the organisms.

Three toxicant-related assumptions have been incorporated into our toxicant models. First, the organisms encounter the toxicant in a contagious, clumped distribution. Because uniform application of the toxicant is unrealistic and the organisms themselves are not evenly distributed, this assumption seems straightforward. A Poisson distribution is used to represent the probability of the clumped organisms interacting with the clumped toxicant. Second, organisms encounter an equal amount of bioavailable toxicant during an iteration. No individual organism encounters more toxicant than any other. The effective dose determines the response based on

the dose response curve for the toxicant. The dose response curve is based on that of the modeled chemical taken from laboratory data, using mortality as the response endpoint. A constant effective dose guarantees a consistent response during each iteration. Therefore, if the response is 50% mortality, half of the organisms that encounter the toxicant will die and half will experience no effect. Death is immediate. Third, the only disturbance in the model is from the toxicant. No other stochastic events, such as storms or diseases, are considered.

Types of toxicants modeled

Two basic toxicant metapopulation models are discussed in this paper, each representing a different type of toxicant. The first model describes persistent compounds such as metals, radioactive materials with long half-lives, and other chemicals that can remain in a system without significant degradation. The effective dose of the persistent toxicant remains constant throughout the run of the model. The second model looks at chemicals that degrade at a constant rate over time, such as organophosphates. A constant percentage of the remaining toxicant is removed at each iteration of the model. A Poisson distribution and the subpopulation size of the dosed patch determine the exposure and the amount of toxicant metabolized at each iteration.

Patch arrangement and terminology

Two types of patch arrangements were used to investigate the questions of how a toxicant can affect different patches. The three-patch circular model allows immigration to occur directly between all three patches (Fig. 1). The three-patch linear model removes immigration from between patches 1 and 3. A distance factor allows the investigation of different distances between patches by reducing immigration with increased distance. Distance XY can simulate barriers for migration from patch X to patch Y.

Information on how to acquire an example program for a linear, degradation model is listed in Appendix A as well as a listing and description of selected variables and the dose-response and growth curves for three patch metapopulation models.

In the presentations that follow, subpopulations 1, 2, and 3 reside in patches 1, 2, and 3, respectively. Patch 1 is always the contaminated patch and always on one end of the linear model. Patch 1 is a source patch if its subpopulation is above the minimal viable population. Time is in iterations, although the iterations represent years for calculations of reproductive and migratory rates of organisms and the degradation of toxicants.

Distance between patches

The influence of distance on migration rate is represented in the model by:

$$d_{ij} = N_i * H_j / D_{ij}. \qquad (5)$$

Here, the migration rate d_{ij} from patch i to patch j is dependent on the number of individuals in patch i, the habitat H_j available in patch j, and the distance D_{ij} between patch i and patch j. The greater the distance, the less the exchange of individuals between subpopulations. Conversely, the smaller the distance, the greater the exchange. This formula is different from that used by Levins [16], who assumes an exponential relationship between the migration rate and the distance, but emphasizes that it is not known how the migration rates between patches change with the distance. Sherratt and Jepson [19] found a linear relationship between the distance between plots and migration into a plot for carabidae following exposure to dimethoate.
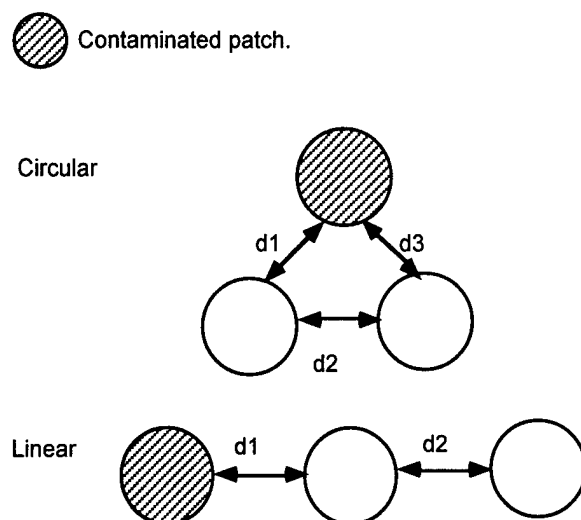
Figure 1. Schematic diagram of the metapopulation models. In the circular model and with all of the distances the same, each patch is equidistant from the other. In the linear model, the ends are twice as far from each other compared to the middle patch.

Computation

The baseline model was tested using the input parameters of Wu, Vankat, and Barlas [18] and the results compared. The output without a toxicant was the same as previously reported.

The time interval for calculation in the model was set at 1.0 to correspond to the same length of time as the generation time of the organisms. In these models it is assumed that the organisms do not respond more rapidly to changing conditions than one generation interval. This assumption does mean that in certain circumstances the populations will oscillate because of the lag in the response time.

The circular and linear nondegradative models were run at least 10 times each to compare the results. The degradative models exhibited several discrete types of outcomes. To estimate the probabilities of each outcome the models were run approximately 300 times with the same initial conditions. Output from the models was recorded in tabular and graphical form. When some of the models exhibited only a few discrete final outcomes, each run was classified according to patterns of extinction and population dynamics.

Results

Persistent toxicant model

The results from the persistent toxicant models demonstrate that the effect of the toxicant can be spread to other patches. When patch 1 is dosed with any amount of chemical, the toxicant death makes more habitat available for immigration from the other patches. The increased immigration reduces the emigrant subpopulations from the nondosed patches, producing a population reduction in the absence of a toxicant in those patches.

Typical output from a circular model is presented graphically in Fig. 2. Replicate runs reveal different trajectories because of the stochastic nature of the exposure of a toxicant to the organisms. However, the general patterns discussed below do hold. A LD_{50} in the dosed patch lowers the subpopulations in all three patches. None of the subpopulations reach the carrying capacity of the patch. Also note that with an LD_{50} it is impossible to tell which of the patches is dosed. The reduction in all three populations due to an LD_{100} compared to the carrying capacity or the 50% mortality case is clear. Fluctuations in the population numbers in the three patches are also more abrupt. However, organisms do exist in the dosed patch, which is the result of migration from the other patches.
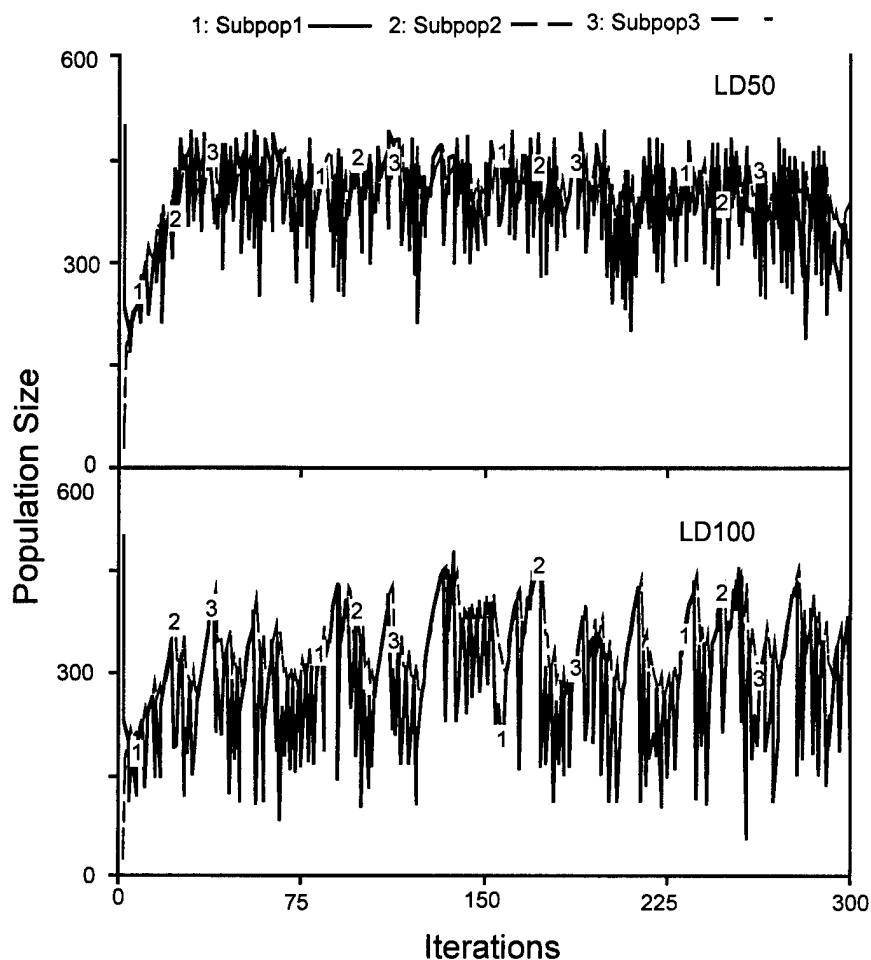
Figure 2. Circular model metapopulation dynamics. Initial conditions 499, 20, and 40 for patches 1, 2, and 3, respectively. Distance is equal to 1. At a contamination level equal to the LD_{50}, all three populations fluctuate dramatically and appear to be tightly coupled. At a higher level of contamination, the LD_{100}, the amplitude of the oscillations increase, but note the difficulty of separating the dosed and nondosed subpopulations.

In the circular model with all distances between patches equal, subpopulations 2 and 3 track each other after the population sizes of the patches converge. Both patches respond identically to the effect on patch 1 because the immigration and growth rates and other patch specific variables are all equal. The length of time to convergence depends on the initial conditions and the Poisson distribution determining the early effect of the toxicant.

Output from the linear model differs significantly since patch 3 is not directly connected to the dosed patch (see Fig. 1). Typical output from the model is displayed in Fig. 3. A dose equal to the LD_{05} does produce irregularities in the dosed patch, but the population size is only slightly reduced. A dose equal to the LD_{50} does reduce the population numbers compared to carrying capacity. The population in the dosed patch fluctuates wildly compared to the other patches. The effect is also moderated by distance, because subpopulation 2 sees a greater reduction in size than subpopulation 3. Although subpopulation 1 is exposed to a dose of 50% mortality, several times during the simulation the population size is the same as the other two populations. In other instances, subpopulation 1 almost becomes extinct. An LD_{100} does not cause extinction of subpopulation 1 in the affected patch. Again subpopulation 1 is rescued by the other uncontaminated patches. Compared to other cases, the amplitude of the fluctuations of

subpopulation 1 is greater, yet the size of subpopulation 1 occasionally converges with that of the nondosed patches. Extinction, as in the elimination of all organisms in the nondosed patch, does not occur, although the individuals are predominantly from subpopulations 2 and 3. Also note a further reduction in the sizes of subpopulations 2 and 3 in the LD_{100} simulations.
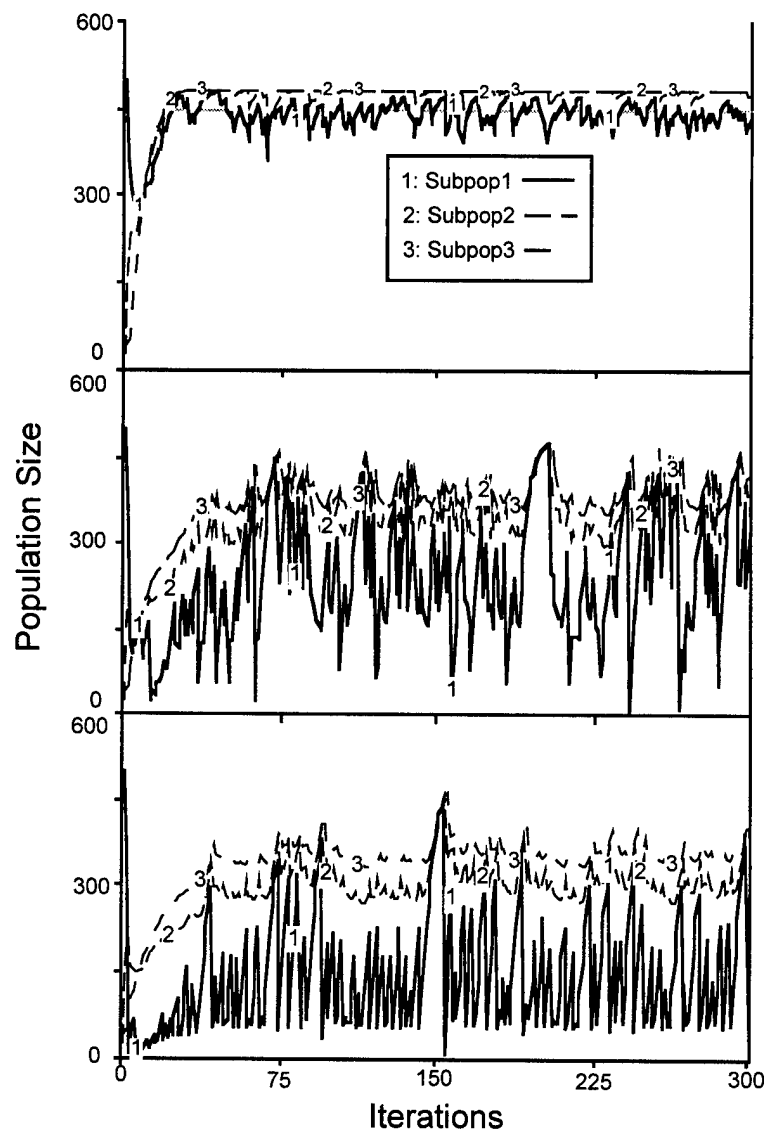
Figure 3. Linear model metapopulation dynamics. Initial population sizes are 499, 20, and 40 for patches 1, 2, and 3, respectively. At LD_{05}, erratic fluctuations are noted in subpopulation 1, but the other subpopulations appear unaffected. At a constant effective dose at LD_{50}, the fluctuations in all of the populations are dramatic. At a constant effective dose at LD_{100}, dramatic fluctuations in subpopulation 1 occur, at times even equaling those of the nondosed patches.

Distance between patches also has an effect on the dynamics of the metapopulation simulation. In Fig. 4, a three-patch linear model, all of the subpopulations begin with an initial size of 100 and patch 1 is dosed with a toxicant at 100% mortality. The distance between the patches ranges from 0.5 to 5 units in the three simulations. At a patch distance of 0.5 units, the three patches are tightly coupled, with large fluctuations in population size and often a confluence in number of all three populations. Due to the proximity of the patches, the nondosed patches serve as a refugia for subpopulation 1, but at the same time the nondosed patches are subject to major variations in population sizes. At a patch distance of 1 unit, subpopulation 1 is still subject to large fluctuations, but subpopulations 2 and 3 demonstrate much less variability than before. The population size of subpopulations 2 and 3 is still less than the carrying capacity, which reflects the impact of the toxicant in patch 1. As the distance increases to 5 units, no variation is noted in subpopulations 2 and 3. Subpopulation 3 is very near carrying capacity and subpopulation 2 is still slightly depressed. In comparison, subpopulation 1 is now consistently lower than that at the other distances and fluctuations are less. The rate of migration from the other patches is now much less, keeping subpopulation 1 quite low. Note, however, that animals still exist in patch 1, even with mortality of 100%.

Toxicant degrading over time

The output from the toxicant degrading over time revealed three basic types of outcomes from the identical initial conditions (Fig. 5). In the three-patch linear model case illustrated, the source patch is dosed with an initial concentration equal to the LD_{100}, and degradation occurs until on the 60th iteration when the compound is virtually eliminated from the patch. In the majority of cases, all three subpopulations reach the carrying capacity of the respective patches [Fig. 5(a)]. However, in a certain number of cases, subpopulation 1 becomes extinct, and the other two subpopulations exist at the MVP [Fig. 5(b)]. In a third case, all three subpopulations exist at the MVP for the duration of the experiment [Fig. 5(c)]. Note that the population level effects of the toxicant persist long after degradation has eliminated the toxicological impacts.

In some of the combinations of distance, initial population size, and toxicant loading, the populations do not attain a steady state condition but exhibit a regular bifurcation in the dynamics of the subpopulations. Figure 6 compares the dynamics of two systems identical except for patch distance. Initial population size is 500, which is the carrying capacity, in each case. At a patch distance of 0.8 units, all three populations exhibit a regular oscillation with each subpopulation having a different amplitude. After approximately 200 iterations, the oscillations become regular and persist after the degradation of the toxicant. After increasing the patch distance to 1 unit, the bifurcation does not exist. The shorter the patch distance, the more likely the population will exhibit a bifurcation of the dynamics. Because the patch distance was very short, the assumption of the population responding no faster than one generation interval was altered. If the response time of the population was decreased from 1.0 to 0.5, the oscillations became rare and not persistent.

The initial population size of the subpopulations also can alter the number of available categories of outcomes and their frequency. Table 1 summarizes the output from a three-patch circular metapopulation model, at a patch distance of 6 units and a carrying capacity of 500. The initial toxicant concentration and rate of degradation are the same as in the other simulations. The frequencies are based on 50 simulations, each with the same initial conditions.
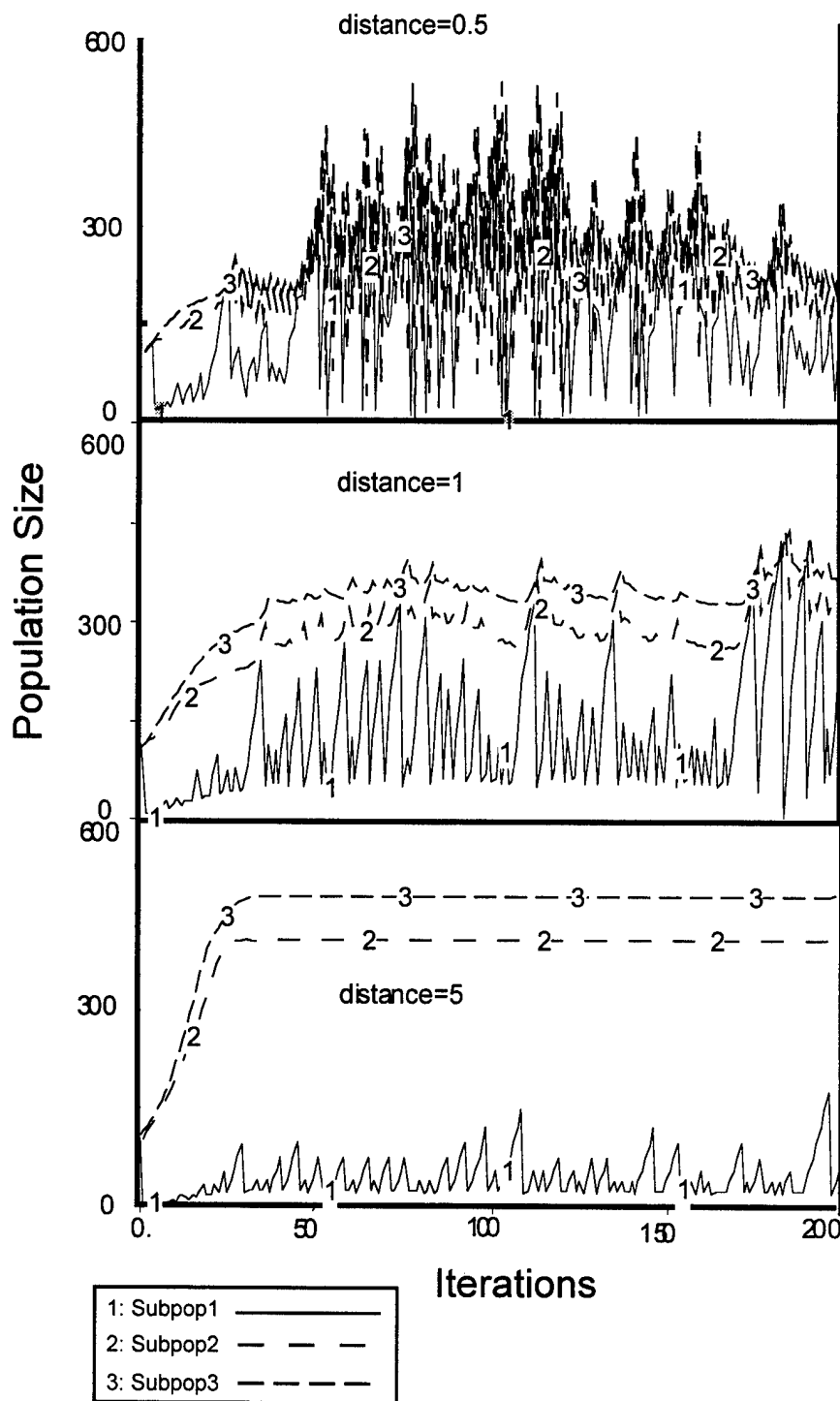
Figure 4. Importance of patch distance on subpopulation dynamics. In the persistent toxicant model at LD_{100}, an increase in distance decreases the amplitude of the oscillations of all subpopulations. At a distance of 0.5 between the three patches, the amplitude of the oscillations is dramatic. Often all three subpopulations overlap. At a distance of 1, subpopulation 1 is still highly variable, but the amplitude of the oscillation is reduced in the other subpopulations. At a distance of 5, the other subpopulations do not reflect the oscillations occuring in subpopulation 1. Note that subpopulations 1 and 2 are still below carrying capacity.
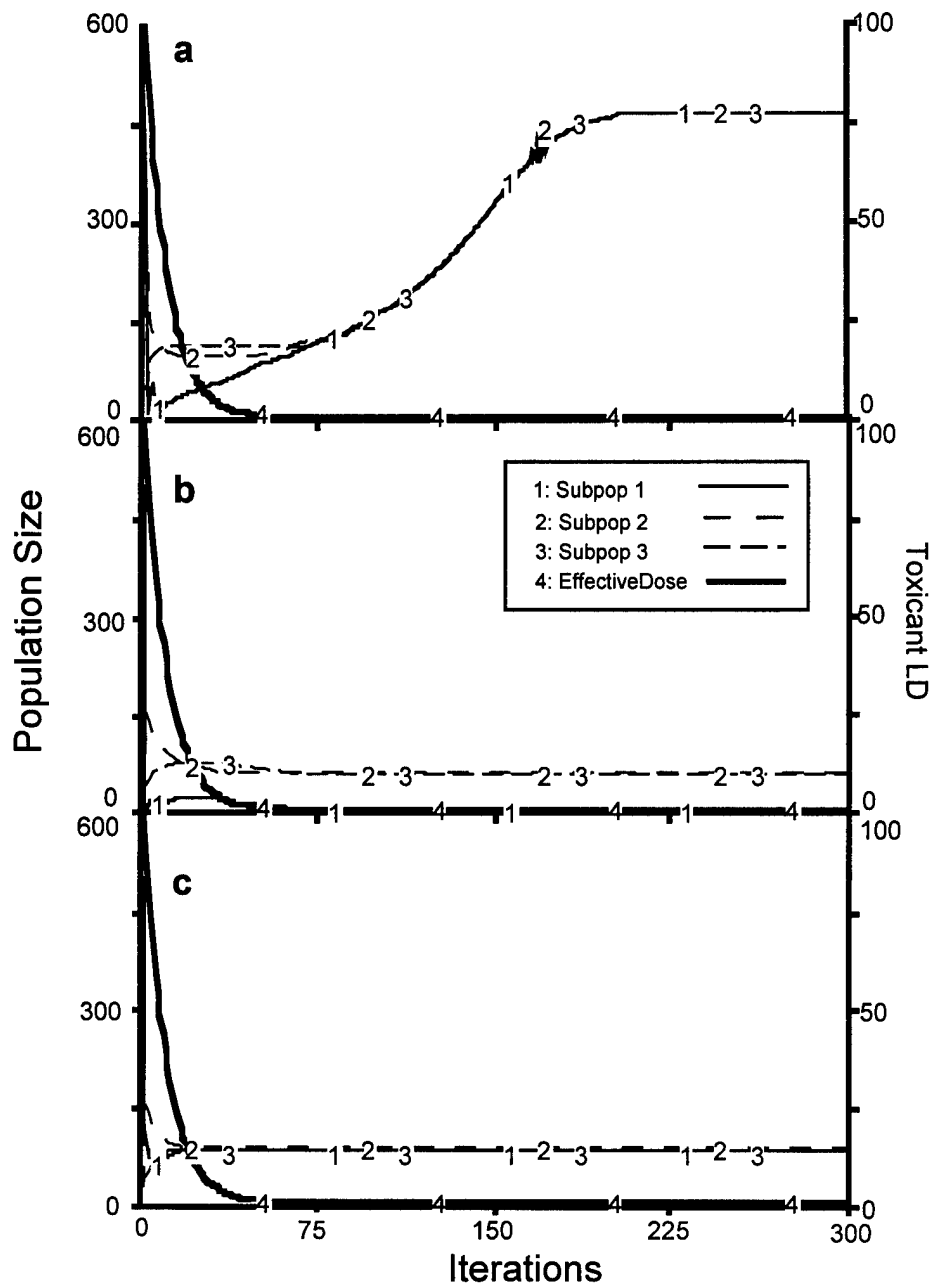
Figure 5. Outcome types from a linear degradation toxicant model with the initial conditions of 499, 20, 40, distance of 1 between the subpopulations, and an initial effective dose of an LD_{100}: (a) 67% of the runs resulted in all 3 subpopulations reaching carrying capacity; (b) 18% of the runs resulted in population 1 going to extinction and populations 2 and 3 staying near MVP; (c) 15% of the runs resulted in all three subpopulations staying near the MVP.
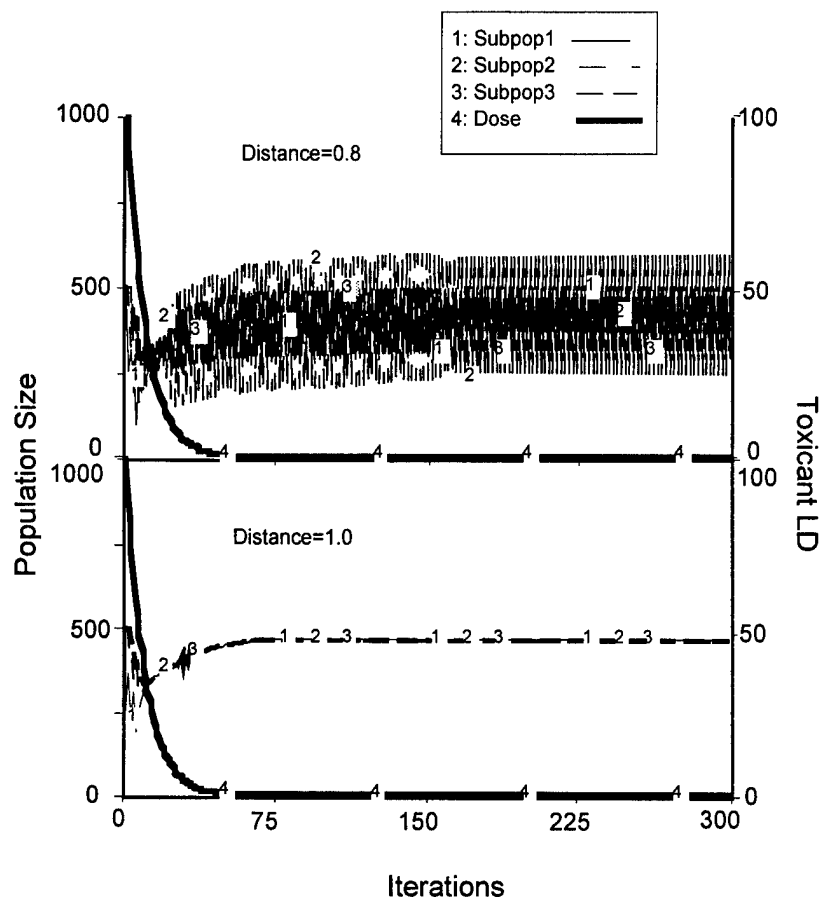
Figure 6. Oscillations in the dynamics. A combination of large initial population size and short distance between patches can produce oscillations in all populations. With initial population size 500 and distance between patches 0.6, oscillations result. The shorter the distance, the greater the oscillations. When the initial population size is 500 and the distance is 1, small oscillations are seen in the initial generations, but all subpopulations reach the range of CC.

Table 1. Range and frequency of outcomes for a circular degradative model with a distance equal to 6 between patches.

Initial subpopulation 1 size	Percent of the outcomes		
	1m2m3m	1c2c3c	1b2b3b
100	80	20	0
120	22	72	2
140	0	82	18

1m2m3m = All populations are at the MVP.
1c2c3c = All populations are at carrying capacity.
1b2b3b = All populations are demonstrating bifurcated dynamics.

At an initial population size in the three patches of 100, two categories of outcomes are possible. In 80% of the cases, the three subpopulations remain at approximately the MVP. Twenty percent of the simulations result in all three subpopulations reaching the carrying capacity of the patch. Increasing the initial population size to 120 in all patches results in three

outcomes. Twenty-two percent of the outcomes result in all three subpopulations staying at the MVP, and 72% of the time all subpopulations reach carrying capacity. A third outcome is introduced, and 2% of the outcomes exhibit a bifurcation in the dynamics of all three subpopulations. Another slight increase in initial population size, to 140 individuals in each patch, exhibits a new pattern. This set of initial conditions results in two outcomes. In 82%, of the simulations all three populations reach carrying capacity. In 18% of the simulations all three populations exhibit bifurcated dynamics. Slight differences in the initial population sizes change the number and frequency of the outcomes of this model.

The pathways of the trajectories within these simulations can be highly variable. Figure 7(a) portrays the first 20 trajectories of subpopulation 1 in a linear degradation model with initial population sizes of 499, 20, and 40 in patches 1, 2, and 3, respectively. Three categories of outcomes are possible for subpopulation 1: extinction, reaching MVP, or reaching carrying capacity. However, each outcome is reached in a different trajectory. Sometimes carrying capacity is reached early in the simulation, at other times carrying capacity is not reached until after more than 200 iterations. Figure 7(b) plots the variance and mean of these 20 simulations. Note how the variability increases over time when multiple outcomes are available. If only one outcome is available (usually reaching carrying capacity), variance reaches a maximum and then reduces to zero as each trajectory reaches the steady state. Different patterns in variance over time occur with differences in the number and type of outcomes.

The type and frequency of outcomes in the degradative models depend on the arrangement of the patches, the distance between patches, and the initial population sizes. The effects of these variables on the frequency of an outcome for a three-patch linear model are presented in Table 2. In Table 2 the outcome charted is the frequency of subpopulation 1 staying above the MVP during the entire simulation. This measurement is important because if a subpopulation decreases to below the MVP, it will have to be rescued by the other uncontaminated patches. Distance between patches and initial population size in the three patches are the dependent variables.

At low initial population sizes and short patch distances, the probability of the subpopulation staying above the MVP is low in the degradative models. At each initial population size, an optimum patch distance exists beyond which the probability of staying above the MVP decreases. At an initial subpopulation size of 100, the chance of staying above MVP is much better, and the optimum patch distance is 0.4 units. At an initial subpopulation size of 250, the chance of staying above MVP increases to 100% at a patch distance of 0.4 to 3 units. Below 0.4 units, the probability is zero. A further shift occurs as the initial population size is set at 500. The chance of staying above the MVP is 100% at patch distances between 0.4 and 6 units.

The data from the simulation can be examined for other probabilities depending on the endpoint of interest. The probability that subpopulation 1 does not become extinct at any time can be considered [Fig. 8(a)]. In this instance the genetic information unique to the subpopulation is lost if not residing due to outbreeding to the other subpopulations. Note that extinction during some part of the simulation does not translate to the lack of a subpopulation 1 at the end of 300 iterations. Figure 8(b) shows that although extinction may have occurred, more often patch 1 has been recolonized and a population does exist in that patch. As noted above, large initial population sizes at a greater distance from the contaminated patch increase the chances of a population being found in patch 1.

Table 2. Importance of initial population size and patch distance. The matrix is the percentage of trials where the size of subpopulation 1 did not decrease below the MVP. The model is a three-patch linear with degradation of the toxicant.

Patch distance	Initial population size subpopulation 1				
	54	75	100	250	500
0.1	0	12	68	0	4
0.4	0	16	92	100	100
0.6	0	8	32	100	100
1.0	0	1	16	100	100
3.0	0	4	28	100	100
6.0	0	0	24	84	100
10.0	0	0	8	88	96
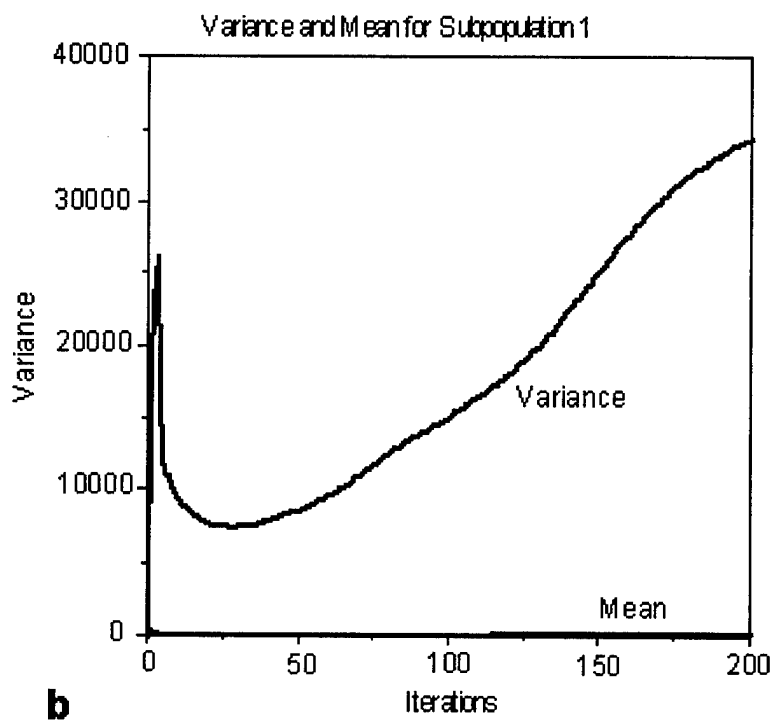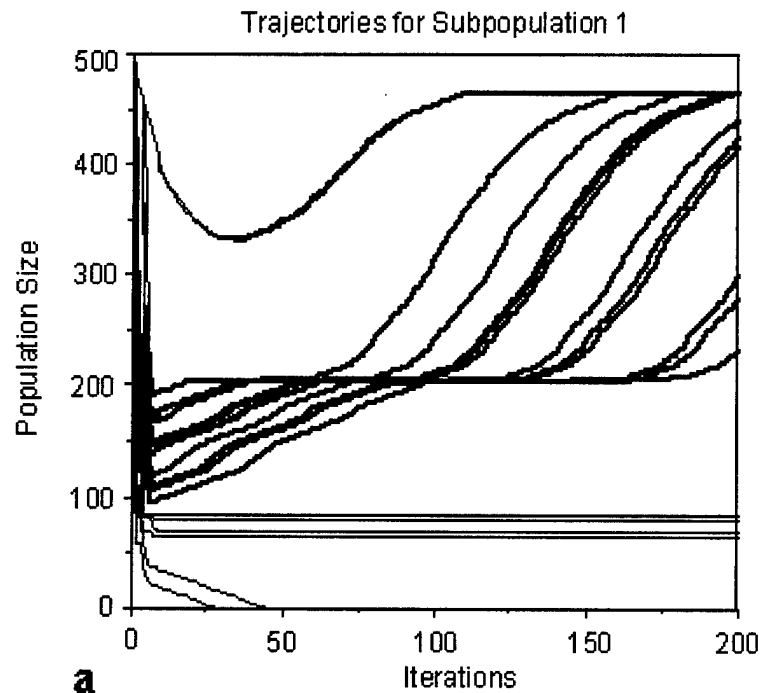
Figure 7. Variation in trajectories: (a) Although only three different outcomes are possible, each trajectory is unique; (b) In these simulations the variance between runs increases over time until a maximum is reached. Initial conditions: subpopulation 1 = 499, subpopulation 2 = 20, subpopulation 3 = 40, distance = 10.

Probability that Subpopulation 1 is not extinct at any time

a
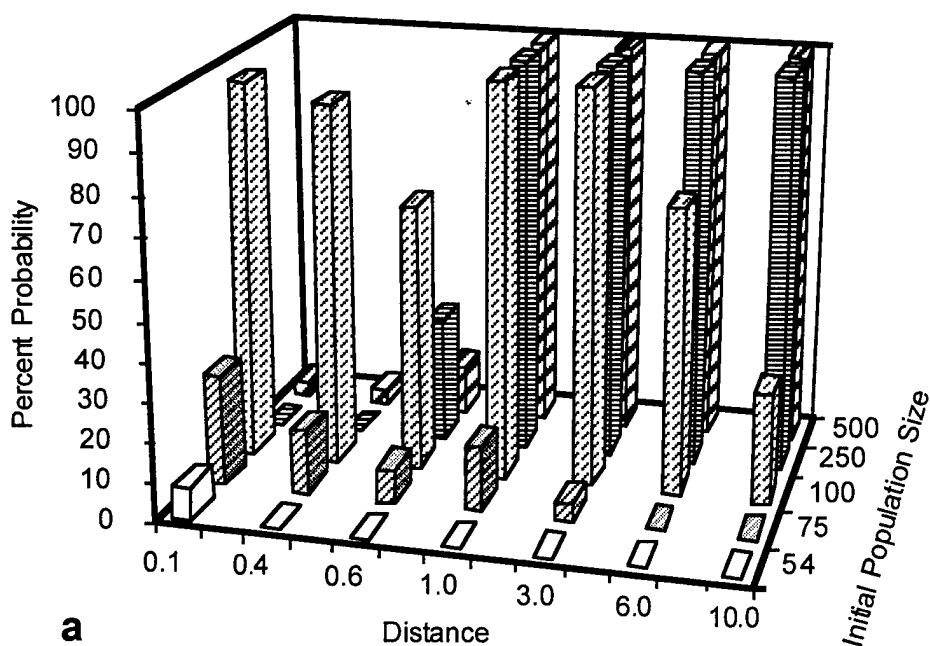
Probability of Subpopulation 1 being alive after 300 generations

b
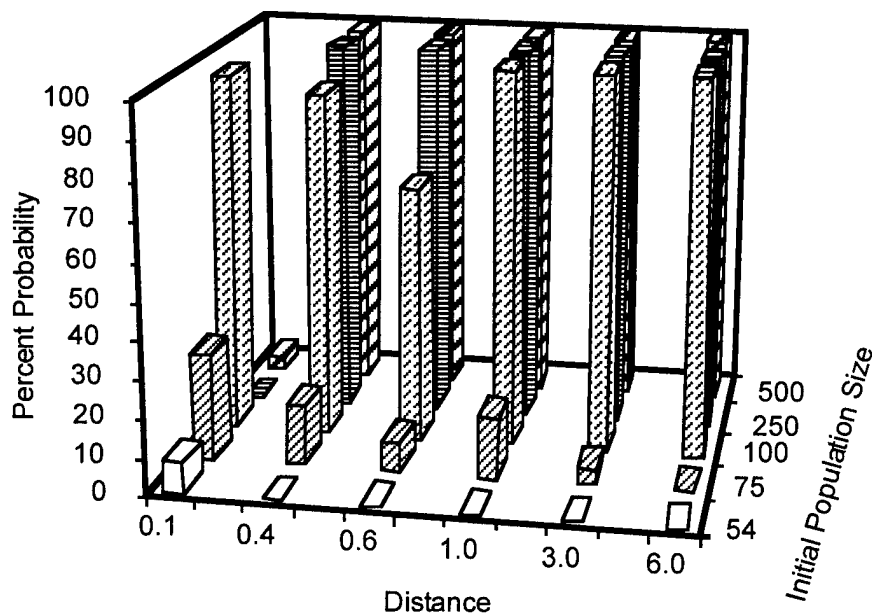
Figure 8. Frequency of outcomes in a three-patch linear degradative model: (a) The frequency of subpopulation 1 does not become extinct at any time during the simulation; (b) portrays the frequency of a population existing in patch 1 at the end of 300 iterations.

Discussion

Simulation studies cannot replace experimental or field studies, but these simulations do raise interesting questions central to the prediction of impacts due to chemical stressors. The output from these simple models indicate that landscape features such as patch arrangement and distance can play a critical role in determining the impacts of chemical stressors. These findings corroborate using a different approach several of the conclusions of Sherratt and Jepson [19], and Maurer and Holt [20] on the importance of dispersal rate and arrangement of the landscape pattern on the impacts of chemical stressors. Impacts due to chemical stressors may also spread to subpopulations that have never been directly dosed, and these impacts can continue after the degradation of the stressor. This effect is action-at-a-distance.

Action-at-a-distance states that the impact to one subpopulation can be transmitted by immigration to other patches within the metapopulation. An impact can occur to a nondosed subpopulation without any direct contact of any organism within that patch to the toxicant. In other words, the dynamics of a subpopulation can be altered without exposure to the stressor. In the case of persistent toxicants, the impact on the nondosed subpopulations was a change in population dynamics and maximum density within a patch. The alterations were persistent, and for close patch distances, the fluctuations dramatic. In the degradative models, a variety of outcomes were available and persisted after the degradation of the toxicant. If these models are representative, a lack of a measured toxicant in an environment does not represent the absence of an extant impact; the stressor may have been historical and the effects manifested by the current population dynamics.

Using the degradative model structure described above, many different categories of outcomes were available from the same set of initial conditions. These categories often involved different combinations of species reaching carrying capacity, reaching MVP, or becoming extinct. Oscillations in the trajectories of the populations were also possible. The probabilities of these outcomes could be computed, but no way exists of computing a priori the trajectory of any particular simulation. This is due to the stochastic nature of the exposure to the toxicant within subpopulation 1. The trajectory of each simulation was therefore different, even if the final outcome was limited to a few categories. In some of the combinations of initial conditions, the differences in outcomes were the maximum possible in these types of models: reaching carrying capacity or extinction. The same set of initial conditions that could produce the survival of all three subpopulations could also produce extinction of one or more of the subpopulations, depending on the chance exposures of organisms in subpopulation 1.

The models also have regions where small differences in initial conditions can result in marked changes in the probabilities of outcomes. Table 2 provides an example where changes in the initial size of subpopulation 1 drastically alter the frequency of the final outcomes. Table 2 demonstrates the dramatic changes in the probability of an outcome that occur with different combinations of initial population size and patch distance. In attempting to predict the impacts of stressors to metapopulations, the initial population size and effective distance between patches is critical in estimating possible categories of outcomes and their frequency. If these models are a rough guide, estimating a small population within a range of ±10% will not be sufficient to predict outcomes due to toxicant stress.

Fallacy of control and reference sites

The most important conclusion derived from these simulations is that a nondosed patch is affected by the toxicant in the dosed patch. As long as immigration connects a subpopulation to a dosed patch, an effect occurs. Increasing the migratory distance or placing barriers between the patches may reduce the magnitude of the effect. This result has important implications in field research, especially to the construct of the reference site. A patch connected by immigration, via any route, to the dosed patch is unsuitable as a reference site. The site is impacted by the toxicant even though no toxicant is present in the patch. Fluctuations in the reference site that might be described as natural variation can be due to the connection to a dosed subpopulation. Therefore, a reference site cannot be connected to the investigation site. But if a reference site

is far enough away to be unconnected by immigration, will it be a good match for the dosed site and will it properly represent the system being studied? We think not.

Taking experimental replicates from a single site is also not plausible if the populations from each area are connected by migration. Each site, including upstream and downstream sites in an aquatic environment, is connected by migration and, therefore, responding to the stressor. Each sample is not an independent trial. As seen in the simulations, the best indication of which patch is contaminated is found by observing the patterns of the populations. Although connected, each patch has its own dynamic that can indicate the presence of the stressor.

Reconstruction of ecological structures

Recovery of ecological structures to a prestressor state is not possible [21-23]. However, assembly of an ecological structure following a severe disturbance is well documented. Explosions such as those from Krakatoa and Mt. St. Helens often leave huge tracts of land virtually sterilized. Severe chemical contamination can also cause massive disruptions. Assembly of a new ecological structure following such disturbances is particularly dependent on colonization from surrounding resource patches. Those patches closer in migratory distance will interact with the impacted site. These models indicate that the reassembly of these ecological structures will also impact the source patches with ramifications for other adjacent patches. In addition, the outcome is not always fixed. Several different classes of outcomes may occur, each with a variety of trajectories. If we rely on such dynamics to assemble an ecological structure following a pesticide application, we also must accept the varied nature of the assembly process and the spread of the impact to adjacent nondosed populations.

Experimental tests

The following experimental hypotheses have been raised for further investigation to confirm the predictions made by this model. These are based upon the conditions of the model, in which an MVP exists and the growth rate is negative below the MVP.

1. An effect on a subpopulation's size and dynamics will occur in connected, nondosed patches.
2. Nondosed patches in a linear metapopulation will show less effect from a toxicant added to an end patch than a circular metapopulation with the toxicant added to any patch.
3. Identification of the dosed patch is possible by analyzing the pattern of the dynamics of the subpopulations.
4. Multiple outcomes can occur from the same set of initial conditions.

These features can be tested in the laboratory by using resource patches linked together by an appropriate bridge. One of the resource patches can be dosed with toxicant and the population dynamics followed. Given appropriate genetic or physical markers, migration and differential mortality can be observed.

Field studies can also be used to test these hypotheses. Instead of only observing a contaminated site, other sites at varying migratory distances from the contaminated must also be sampled. A gradient of population dynamics should be observable, with the greater impact occurring in the subpopulations closest to the contaminated site. These studies could be conducted in an experimental field or as an adjunct to the investigation of a contaminated site.

Conclusions

1. Mortality in one subpopulation has ecologically significant effects on nondosed subpopulations. We term this hypothesis action-at-a-distance, because no direct contact with a toxicant has occurred except in the dosed patch.

2. Because uncontaminated sites connected to the contaminated site by migration of the biota are affected, these sites cannot be reference sites.

3. The arrangement of the patches is critical to the dynamics of the system and the overall impact of a toxicant.

4. Due to the contagious distribution of the toxicant and the stochastic function describing exposure-effect, multiple discrete outcomes often are possible from the same initial conditions. These outcomes can range from extinction to the reaching of the carrying capacity for a patch.

5. If sufficient cleanup is not possible, isolating the contaminated patch may be necessary to allow the formerly connected patches to regain more typical population dynamics.

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Appendix A. The dose response and growth curves for three-patch metapopulation models are presented in Figure 9(a) and 9(b), respectively. The programs written in Stella II can be obtained by emailing landis@cc.wwu.edu. Typical variables used in the toxicant models are provided below.

ActPCNGR#	Actual net growth rate for patch #.
CarryingCap#	Carrying capacity of main patch #.
Crowding_#	The density dependent crowding of patch #.
degrate	The degradation rate of the toxicant, defined by the type of model.
Doseprob #	The probability that an organism will encounter the toxicant.
DoseResponse#	Dose response curve of toxicant, mortality endpoint.
EffectiveDose #	The effective dose of the toxicant in the patch, which is used to determine the response.
Fitness #	Reproductive fitness of the patch, decreases when patch exposed to toxicant relative to the proportion of individuals that come in contact with the toxicant.
Generationtime	Number of iterations the model runs for each generation of the organism.
Habitat_Avail_#	Amount of habitat in patch # available for colonization.
ImR##*	Number of individuals immigrating from patch # to patch #.
InitDose#	The initial dose of the toxicant in the patch.
NetGR#	Net growth of patch # of each generation.
PCNGR#	The per capita net growth rate curve for patch #, based on crowding.
Pois#	Multiplier representing the Poisson distribution as a proportion less than 1.
Response #	The response from the toxicant, i.e., percent mortality.
Subpop#	Total number of organisms in patch #.
ToxDeath#	The number of organisms in patch # killed by the toxicant each generation.
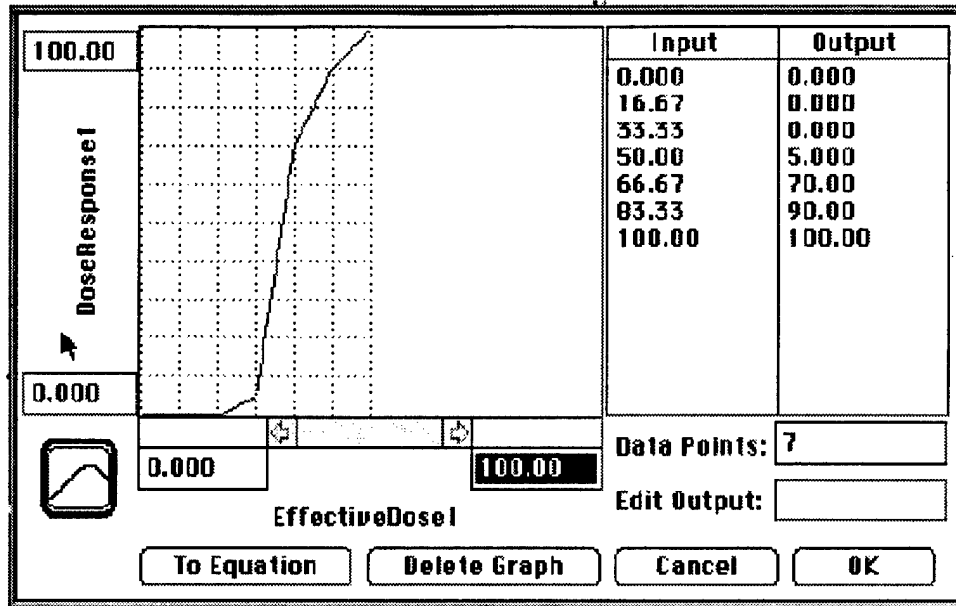
a
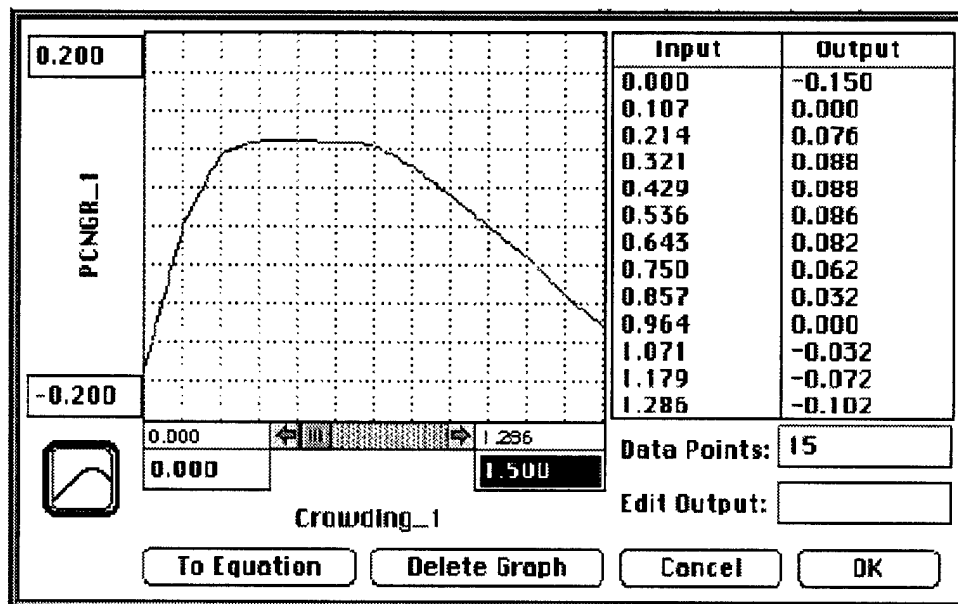
b

Figure 9. The (a) dose-response and (b) growth curves for three patch metapopulation models.